THIN-LAYER CHROMATOGRAPHIC STUDIES OF POLLUTANTS WITH MICELLAR AND NON-MICELLAR SOLVENT SYSTEMS

ABSTRACT

THESIS

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BY

NAHED JABEEN

UNDER THE SUPERVISION OF

DR. ALI MOHAMMAD

DEPARTMENT OF APPLIED CHEMISTRY
FACULTY OF ENGINEERING & TECHNOLOGY
ALIGARH MUSLIM UNIVERSITY
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LIST OF PUBLICATIONS

1. Thin–layer chromatographic studies on mobility of some pesticides through different soil containing static flat–bed phases, A. Mohammad, I.A. Khan and N. Jabeen, *J. Planar Chromatogr.–Mod. TLC (Hungary)*, **14** (2001) 283.

2. TLC studies and separation of heavy metal cations on soil amended silica gel layers developed with surfactant–mediated solvent systems, A. Mohammad and N. Jabeen, *Indian J. Chem. Technol.*, **10** (2003) 79.

3. Soil thin–layer chromatography of heavy metal cations with surfactant–modified mobile phase: separation of coexisting Zinc (II), cadmium (II) and mercury(II), A. Mohammad and N. Jabeen, *J. Planar Chromatogr.–Mod. TLC* (Hungary)

4. Separation studies of transition metal ions with cationic micellar eluents by normal phase thin–layer chromatography, A. Mohamad, V. Agrawal and N. Jabeen, *Chromatography* (Japan),

5. Use of surfactant as extractant for separation of Cr^{6+} from Cr^{3+} and associated metal cations, A. Mohammad and N. Jabeen, *Analusis* (France).

6. Reversed–phase chromatography of metal cations, phenols and amines on silica layers impregnated with tributyl phospate using surfactant–mediated mobile phase systems, A. Mohammad and N. Jabeen, *Acta Chromatographica* (Poland). Accepted.

ABSTRACT

The work embodied in this thesis is related to the application of thin–layer chromatography (TLC) in the analysis of organic (pesticides, amines and phenols) and inorganic (heavy metal cations) pollutants. As an extension to the existing knowledge, several novel, cost–effective and readily applicable chromatographic systems have been identified for TLC analysis of amines, phenols, pesticides and heavy metal cations. The results presented in the thesis contribute substantially to the advancement of normal–phase and reversed–phase TLC procedures. The interesting features of the present study include:

- Use of commercially available silica gel, alumina, kieselguhr and cellulose sorbents as layer material along with precoated high–performance thin–layer chromatographic plates.
- Use of layer materials that are less familiar in TLC. For example, plain soil and soil mixed with silica layers for separation and identification of pesticides and heavy metal cations.
- Use of surfactant as one of the components of mobile phase (e.g. aqueous micellar solutions) to investigate its role in modifying the retention pattern of analytes on TLC plates.
- Understanding about the efficacy of surfactant–mediated mobile phases in respect of enhanced separation selectivity of analytes.
- Utilization of analytical potential of cationic surfactant (N–cetyl–N,N,N–trimethylammonium bromide) containing eluents as a promising medium for chromatographic separations of heavy metal cations.
- Examination of role of inorganic and organic additives in micellar mobile phases on the mobility sequence of analytes (metal cations).

- Identification of most reliable TLC systems for achieving rapid separations of amines, phenols and heavy metal cations and realization of analytically important separations of coexisting ionic as well as non–ionic species.
- Application of the proposed methods for detection, identification and separation of analytes from a variety of spiked samples and semi–quantitative estimation of important species.

The present work formulated with the objective of developing new TLC systems comprising of novel mobile phases and support materials for acquiring improved separations of organic and inorganic compounds has been encapsulated in the form of seven chapters of the thesis. A brief account of the work done is being summarized below:

CHAPTER I is an introductory part, which summarizes the various important aspects of liquid chromatography including its usefulness in the analysis of organic and inorganic pollutants. A brief description on the importance and hazards of the potent pollutants including pesticides has been provided. Some part of this chapter has been devoted to provide a brief knowledge about occurrence, nature and physico-chemical properties of soil. A comprehensive description on TLC including theory, general practices and complete literature survey of last eleven years on its application to aromatic amines, phenols and heavy metal cations, along with soil TLC (for analysis of pesticides and heavy metal cations) has been provided in this chapter. Besides, scattered information available in literature about the use of surfactant-containing solutions as mobile phase systems in the area of liquid chromatography has been brought together in an organized form.

CHAPTER II deals with the study of chromatographic behaviour of some pesticides on silica gel, soil and mixed soil-silica gel layers developed with (i) aqueous ammonium or sodium salt solutions, with or without added N–cetyl–N,N,N–trimethylammonium bromide (CTAB), (ii) pure organic solvents and (iii) aqueous CTAB mobile phase systems. One interesting

aspect of this study is that pesticides such as phosphamidon and dimethoate migrate through pure soil. In addition of several important binary separations, sulphur–containing pesticides have been successfully separated from pesticides having chlorine, phosphorus, or both phosphorus and sulphur atoms in their molecules.Irrespective of the nature of the sorbent used as layer material, phosphorous-containing pesticides moved faster in several mobile phases.The relative mobilities of pesticides obtained on various stationary phases are presented **(Table 1)**.

Table – 1

Mobility trend of pesticides on various stationary phases used

Mobile phase	CLPS	PHM[a]	DM
M_1	Silica gel> soil = soil + silica gel (3:7) = soil + kieselguhr(3:7) = soil + cellulose (3:7)	Soil + kieselguhr > soil + silica gel> silica gel > soil	Soil + silica gel > silica gel > soil + kieselguhr = soil + cellulose = soil
M_5	Silica gel> soil = soil + silica gel = soil + kieselguhr = soil + cellulose	Soil > soil + cellulose > silica gel	Soil > silica gel [b]> soil + silica gel [c]> soil + kieselguhr = soil + cellulose
M_{12}	Silica gel > soil = soil + kieselguhr = soil + silica gel = soil + cellulose	Soil > silica gel	Soil + kieselguhr [c]> silica gel [c]> soil + silica gel [c] > soil = soil + cellulose

a) PHM could not be detected on other stationary phase
b) Triple spots (RF 0.82, 0.45 and 0.0), RF 0.82 is taken for comprison
c) Double spots (second spot at RF = 0.0 in all cases). Higher RF value is taken for comparison ESN, CMN and FVL remain at or near the point of application on all stationary phases.

CHAPTER III deals with chromatography of ten heavy metal cations on soil, silica gel and soil mixed with silica gel sorbent phases using aqueous solutions of CTAB, sodium dodecyl sulphate (SDS), polyoxyethylene dodecyl ether (Brij–35) and iso–octylphenoxyethoxy ethanol (TX–100) surfactants with or without added urea, nitrate or chloride of ammonium and sodium phosphate as mobile phases. In all, twenty–nine stationary phases and twenty-five mobile phases were used in order to examine the mobility pattern and to find out the best TLC system for the separation of metal cations from their multicomponent mixtures on soil mixed with silica gel layers. Addition of silica gel into soil bed leads to the increase of mobility and facilitates the separation of metal cations. Amongst surfactant solutions used, CTAB at concentration level of 0.5M was found most effective. The analytical potential of CTAB is further improved in the presence of urea. The TLC system comprising of silica gel plus soil (9:1, w/w) as stationary phase and 0.5M CTAB plus 0.6M urea (1:1, v/v) as mobile phase was found to be most optimum for achieving separations of metal ions from their multicomponent mixtures **(Table 2)**. The salting–in–effect exhibited by certain metal ions like Cd^{2}+, Ni^{2+}, Ag^{+} or Hg^{2+} is an interesting aspect of present study.

Table – 2

Experimentally achieved separations of metal ions on soil mixed with silica gel layers developed with mobile phase 0.5 M CTAB + 0.6 M Urea (1:1).

Stationary phase	Separations (R_F)
S_9	Ni^{2+} (0.65) – Cu^{2+} (0.30) – Fe^{3+} (0.05), Ni^{2+} (0.62) – Cu^{2+} (0.3) – Pb^{2+} (0.0), Ni^{2+} (0.65) – Cu^{2+} (0.3) – Fe^{3+} (0.0) – Hg^{2+} (0.99), Zn^{2+} (0.12) – Cd^{2+} (0.6)- Hg^{2+} (0.95), Zn^{2+} (0.18)- Fe^{3+} (0.0) – Ni^{2+} (0.62) – Hg^{2+} (0.92).
S_{10}	Zn^{2+} (0.12)–Cd^{2+} (0.55)–Hg^{2+} (0.95), Ni^{2+} (0.7)–Fe^{3+} (0.03)–Hg^{2+} (0.94).
S_{11}	Ni^{2+} (0.72) – Cu^{2+} (0.21) – Fe^{3+} (0.05), Ni^{2+} (0.70) – Cu^{2+} (0.26) – Pb^{2+}(0.0), Fe^{3+} (0.02) – Cu^{2+} (0.24) – Ni^{2+} (0.68) – Hg^{2+} (0.97), Zn^{2+} (0.11) – Cd^{2+} (0.67) – Hg^{2+} (0.95), Zn^{2+} (0.17) – Fe^{3+} (0.0) – Ni^{2+} (0.63) – Hg^{2+} (0.95), Pb^{2+} (0.0)- Cd^{2+} (0.7) – Hg^{2+} (0.96).
S_{12}	Ni^{2+} (0.61) – Cu^{2+} (0.24) – Fe^{3+} (0.3), Fe^{3+} (0.02) – Cu^{2+} (0.31) – Hg^{2+} (0.99), Ni^{2+} (0.63) – Fe^{3+} (0.03) – Hg^{2+} (0.97), Fe^{3+} (0.02) – Cu^{2+} (0.24) – Ni^{2+} (0.63) – Hg^{2+} (0.97), Pb^{2+} (0.0) – Cu^{2+} (0.31) – Ni^{2+} (0.67) – Hg^{2+} (0.97).
S_{13}	Ni^{2+} (0.71) – Cu^{2+} (0.32) – Hg^{2+} (0.97), Fe^{3+} (0.03) - Cu^{2+} (0.33) – Hg^{2+} (0.97), Fe^{2+} (0.03) – Ni^{2+} (0.68) – Hg^{2+} (0.96), Fe^{2+} (0.02) – Cu^{2+} (0.32) – Ni^{2+} (0.72) – Hg^{2+} (0.97), Zn^{2+} (0.15) – Cd^{2+} (0.65) – Hg^{2+} (0.98), Pb^{2+} (0.0) – Cu^{2+} (0.31) – Ni^{2+} (0.71) – Hg^{2+} (0.97).
S_{14}	Fe^{3+}(0.05) – Cu^{2+} (0.25) – Ni^{2+} (0.65), Fe^{3+} (0.02) – Cu^{2+} (0.35) – Ni^{2+} (0.65) – Hg^{2+} (0.97), Zn^{2+} (0.14) – Cd^{2+} (0.67) – Hg^{2+} (0.96), Pb^{2+} (0.0) – Cu^{2+} (0.32) – Ni^{2+} (0.71) – Hg^{2+} (0.97).
S_{15}	Fe^{3+} (0.02) – Cu^{2+} (0.27) – Ni^{2+} (0.67), Fe^{3+} (0.02) – Cu^{2+} (0.25) – Hg^{2+} (0.95), Fe^{3+} (0.02) –Cu^{2+} (0.26) – Ni^{2+} (0.71) – Hg^{2+} (0.97), Zn^{2+} (0.12) – Cd^{2+} (0.60) – Hg^{2+} (0.91), Fe^{3+} (0.0) – Ni^{2+} (0.66)–Hg^{2+} (0.95), Pb^{2+} (0.0) – Cu^{2+} (0.27) – Ni^{2+} (0.61) – Hg^{2+} (0.95).
S_{16}	Zn^{2+} (0.11) – Cd^{2+} (0.58) – Hg^{2+} (0.98), Fe^{3+} (0.04) – Ni^{2+} (0.60) – Hg^{2+} (0.94), Pb^{2+} (0.0) - Ni^{2+} (0.65) - Hg^{2+} (0.97).

CHAPTER–IV deals with chromatography of metal cations performed on pure soil layers using aqueous solutions of (a) organic and inorganic fertilizers, (b) inorganic fertilizer mixed with potassium, sodium or calcium halide, and (c) sodium dodecyl sulphate (SDS) with added iodide or chloride of K, Na or Ca as mobile phase systems. A novel mobile phase system comprising of 0.001M SDS+NaCl (1:9, v/v) was identified for rapid separation of coexisting Zn^{2+}, Cd^{2+} and Hg^{2+}. The mobility trend of metal cations observed on pure soil layer developed with this mobile phase system was compared with the mobility sequence of metal ions obtained on organic matter free soil layer. A general trend of increase in mobility of metal ions on organic matter free soil layer was noticed. The substitution of anionic SDS by cationic CTAB or a non–ionic (TX–100 or Brij–35) surfactant in the mobile phase hampers the separation of Zn^{2+} from Cd^{2+} and Hg^{2+} . The effect of inorganic additives (sodium molybdate, KSCN) and carboxylic acids (oxalic, tartaric and citric) on simultaneous separation of Zn^{2+}, Cd^{2+} and Hg^{2+} was examined **(Table 3)**.

Table 3

Separation possibilities of Zn^{2+}, Cd^{2+} and Hg^{2+} in the presence of inorganic and carboxylic acids as impurities in the selected mobile phase $M_{26}(a)$

Mobile Phase	R_F values			Remarks
	Zn^{2+}	Cd^{2+}	Hg^{2+}	
$M_{26}(a)$	0.02	0.54	0.85	Good ternary separation of Zn^{2+}, Cd^{2+} and Hg^{2+}
Sodium molybdate + $M_{26}(a)$				
1:9	0.03	0.14	0.85	Binary separation of Hg^{2+} from Zn^{2+} and Cd^{2+}
9:1	0.05	0.04	0.07	No possible separation
Potassium thiocyanate + $M_{26}(a)$				
1:9	0.07	0.52	0.75	Binary separation of Hg^{2+} from
9:1	0.07	0.19	0.74	Zn^{2+} or Cd^{2+}
Oxalic acid + $M_{26}(a)$ *				
1:9	0.21	0.47	0.85	Poor separation possibility of Zn^{2+}
9:1	0.17	0.55	0.79	from Cd^{2+} and of Cd^{2+} from Hg^{2+}
Citric acid + $M_{26}(a)$				
1:9	0.53	0.50	0.70	No separation of Zn^{2+} from Cd^{2+}
9:1	0.70	0.72	0.67	No separation of Zn^{2+} from Cd^{2+}
Tartaric acid + $M_{26}(a)$				
1:9	0.40	0.47	0.89	No separation of Zn^{2+} from Cd^{2+}
9:1	0.51	0.70	0.78	No separation of Cd^{2+} from Hg^{2+}

* ΔR_F (R_F Cd^{2+} - R_F Zn^{2+}) = 0.26

ΔR_F (R_F Hg^{2+} - R_F Cd^{2+}) = 0.24,

For good separation $\Delta R_F > 0.30$ has been considered.

CHAPTER–V deals with the assessment of analytical potential of surfactant–mediated mobile phase systems in thin layer chromatographic separation and identification of metal cations. Effects of (a) concentration of surfactant in the mobile phase (b) acidity or basicity of surfactant–containing developer medium and (c) the presence of electrolyte or non-electrolyte additives in the mobile phase systems on the mobility of metal cations were examined. Comparison of chromatographic performance of hybrid mobile phase systems comprising of micelle (3% CTAB)–water–alcohol (methanol, ethanol, propanol or butanol) over aqueous micellar mobile phase systems was also investigated. Semiquantitative determination of Zn^{2+}, Cd^{2+} and Hg^{2+} by visual comparison of colour intensities on the TLC plates and by measurement of spot area was attempted. Identification and TLC separation of Zn^{2+}, Cd^{2+} and Hg^{2+} from river water, industrial wastewater and sulphide sludge have been done. The method is found well suited for identification and separation of Hg, Zn and Cd in synthetic ores such as cinnabar (HgS), zinc blende (ZnS) and greenoekite (CdS), **(Table 4)**.

Table 4

Recovery and separation of coexisting Zn^{2+}, Cd^{2+} and Hg^{2+} ions from spiked water and synthetically prepared metal ores and heavy metal sludge samples

Stationary phase: silica gel

Spiked/synthetic samples	R_F value		
	Zn^{2+}	Cd^{2+}	Hg^{2+}
River water	0.05	0.46	0.95
Sea water	0.03	0.46	0.95
Sulphides	0.14	0.56	0.96
Distilled water	0.07	0.47	0.95

CHAPTER VI deals with the chromatography of some heavy metal cations performed on high–performance silica gel 60 F_{254} precoated plates using aqueous solutions of cationic, anionic and non–ionic surfactants at concentration levels corresponding to above, near and below their critical micelle concentrations (CMC). Mixed micellar systems comprising of ionic plus non–ionic surfactants were also tested to achieve certain separations of metal ions which were not possible with micellar solution of individual surfactant. Micellar surfactant solutions (cationic, anionic or non–ionic) with added urea were used to illustrate the difference in the chromatographic performance of cationic (CTAB) and anionic (SDS) surfactants micelles.

Separation of Cr^{6+} or Mo^{6+} from associated metal ions like Cr^{3+},Fe^{3+},Cu^{2+} or UO_2^{2+} etc. was achieved using 0.0225 M aqueous micellar solution of CTAB. Mutual separation of Cr^{6+} and Cr^{3+} was investigated at different pH levels of the sample solution. The lower limits of detection of these cations on high–performance thin–layer chromatographic (HPTLC) plates were determined and semiquantitative determination of Cr^{6+} and Cr^{3+} by spot–area measurement was attempted. The proposed method was successfully applied for identification of Cr^{6+} as well as the mutual separation of Cr^{6+} from Cr^{3+} from industrial wastewater sample and synthetic alloys **(Table 5)**.

Table–5

Separation of coexisting Cr^{3+} and Cr^{6+} from spiked industrial wastewater and synthetic alloys on silica HPTLC plates using mobile phase M_4.

Spiked/synthetic samples	Separation (R_F)	
	Cr^{3+}	Cr^{6+} (a)
Industrial wastewater	0.03	0.95
Cobalt alloy	0.03	0.90
Nichrome alloy	0.02	0.93

a) Detection of Cr^{6+} was done by saturated alcoholic solution of $AgNO_3$.

CHAPTER–VII deals with the use of tri–n–butyl phosphate (TBP) as an impregnant in thin layer chromatographic separation of coexisting metal ions, amines and phenols. Silica gel impregnated with 0.001M TBP as stationary phase and aqueous micellar solution of CTAB, a cationic surfactant as mobile phase was found to be the best chromatographic system for obtaining important separations of metal cations (Cr^{3+}–Cr^{6+}, Fe^{3+}–Mn^{2+}–Cr^{6+}, VO^{2+}–Mn^{2+}–Cr^{6+} etc.) and phenols (o-cresol–m-cresol, m-aminophenol-o-aminophenol etc.) from their mixtures. Aqueous solution of non–ionic surfactant, Brij–35 ($8.3x10^{-6}$M) proved to be favourable for achieving good separations of amines (p–dimethylaminobenzaldehyde-L-tryptophan, p–dimethylaminobenzaldehyde-indole, etc.) on 0.001M TBP impregnated silica layers. The effect of metal cations on the separation of amines and phenols was examined. Conversely, the effect of amines and phenols on the separation of metal ions was investigated. A marginal influence of common excipients on the separation of analytes was experienced **(Table 6)**.

Table–6

Separation of amines and phenols in the presence of inorganic impurities developed with mobile phases M_6 or M_{17} on 0.001M TBP impregnated silica layers.

Impurities	Separation (R_F)
Fe^{3+}	p-DAB(0.45) –ID(0.70), p–DAB(0.44) –L-TRYP(0.76), o-Crol(0.74) –m-Crol(0.00), o-Crol(0.74) –p-Crol(0.00), m-Aph(0.70)/p-APh(0.83) –o-APh(0.00).
Cu^{2+}	p-DAB(0.46) –ID(0.71), p-DAB(0.45) –L-TRYP(0.75), o-Crol(0.73) –m-Crol(0.00), o-Crol (0.75) –p-Crol(0.00), m-Aph(0.73)/p-APh(0.84) –o-APh(0.02).
Ni^{2+}	p-DAB(0.47) –ID(0.72), p-DAB(0.45) –L-TRYP(0.74), o-Crol(0.72) –m-Crol(0.00), o-Crol(0.76) –p-Crol(0.02), m-APh(0.72)/p-APh(0.82) –o-APh(0.00).
Co^{2+}	p-DAB(0.46) –ID(0.71), p-DAB(0.45) –L-TRYP(0.74), o-Crol(0.73) –m-Crol(0.00), o–Crol(0.76) –p-Crol(0.00), m-Aph(0.73)/p-APh(0.83)–o-APh(0.00).
UO_2^{2+}	p-DAB(0.48) –ID(0.70), p-DAB(0.46) –L-TRYP(0.75), o-Crol(0.73) –m-Crol(0.00), o-Crol(0.75) –p-Crol(0.00), m-APh(0.72)/p-APh(0.82) –o-APh(0.00).

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Dr. Ali Mohammad
Ph.D., D.Sc. (F.N.A.Sc.)
EDITOR : Chemical & Environmental Research

Faculty of Engineering & Technology, Aligarh Muslim University, Aligarh-202 002, India
54-230 AMU IN (Telex) . 91-571-700920, 700921 (Tel) . 456 (Ext.) . mohammadali4u@rediffmail.com (E-mail)

Certificate

Certified that the work incorporated in this thesis entitled "Thin-layer chromatographic Studies of Pollutants with Micellar and Non-Micellar Solvent systems" is the original contribution of Ms. Nahed Jabeen, carried out under my supervision, and is suitable for submission for the award of Ph.D. degree in Applied Chemistry.

Ali Mohammad.

ALI MOHAMMAD

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NAHED JABEEN

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CONTENTS

CHAPTER-I

General Introduction

1.1 INTRODUCTION

Analytical chemistry is one of the most important branches of chemistry. It is being used for the detection and determination of traces of pollutants in air, water and soil environments. Owing to the great importance of pollution in modern life, the environmental chemistry is becoming more and more relevant. By environment we mean not only our immediate surrounding but also a variety of issues connected with human activity, productivity, basic living and its impact on natural resources such as land, water, atmosphere, forest, habitat, health, energy, resources, wildlife etc. In relation to man, the environment constitutes of air, land, water, flora & fauna because these elements regulate the man's life.

1.2 POLLUTION AND POLLUTANTS

The addition to the environment of any substance at a rate that results in higher than natural concentrations of that substance is termed as "*Pollution*". The more acceptable definition of pollution, given by *Holdgate* states as "*pollution is the introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to living sources and ecological systems, damage to structures or interference with legitimate uses of the environment*". Pollutant is a substance present in nature in greater than natural abundance due to human activity, which ultimately has a detrimental effect on the environment and there from on living organisms and mankind.

Some substances such as radioactive dust and certain pesticides (e.g. DDT) can be relatively long lasting and can disperse readily to the point that they become virtually worldwide in distribution. Such pollutants which include many chlorinated hydrocarbon insecticides and several toxic mercury compounds, may enter biological food chains by being concentrated in the bodies of micro organisms (algae and protozoans) and move upward, as successively larger organisms prey

upon smaller ones. Many serious pollutants are closely and fundamentally bound to vital agricultural or industrial systems or products. Abatement of a given pollutant may involve total redesign of an industry or its product. Pollutants generally affecting the environment may be broadly categorized as inorganic and organic pollutants.

i) Inorganic pollutants consist of inorganic salts, mineral acid, finely divided metals, metalloids or metal compounds, trace elements and organometallic compounds.

ii) Organic pollutants include oxygen demanding wastes, disease causing agents, plant nutrients, sewage, synthetic organic compounds like alcohol, ketones, aldehydes, amines, phenols, organic acids and others.

Many inorganic compounds, especially metal ions, are playing a double role in the physiology of organisms. Some are indispensable for normal life while most of them are toxic at elevated concentrations i.e. they adversely affect the activity and well being of living organisms. Recent years have witnessed an increasing concern for the potential toxic effects of metal ions and other inorganic compounds, which constitute part of the products and the by-products of our technologies. This is especially true for heavy metals because it is not ameliorated by natural phenomena of biochemical decomposition. High concentration of heavy metals in surface water is generally associated with industrial discharges (1,2). Metals containing industrial waste constitute a major source of metallic pollution of the hydrosphere. The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in aquatic environment. The important sources of metal pollution include domestic sewage. (3), geological weathering, mining effluents (4,5), atmospheric sources (6) and urban storm water run off (7,8).

Similarly, most of the organic compounds are suspected to be carcinogenic. The presence of the synthetic organic chemicals in water

imparts objectionable smell and offensive taste, even if present in low concentrations. Accumulation of these chemicals in soil and their subsequent mobilization into the plants has posed a serious concern (9). Organic particulate matter originates from wide variety of sources such as emission from vegetation and automobiles, combustion of fuels etc. Polycyclic aromatic hydrocarbons (PAH) and organic particulate matter are known to have carcinogenic effect. Naturally occurring chelating agents such as humic acids and aminoacids exist in natural water and soil. The properties of water are generally influenced by the humic substances, both soluble and insoluble due to their acid-base, adsorptive and complexing properties. The first International Conference on Earth held in Rio in 1992 may be considered to be the start of awareness for environmental pollution. Environmental education is important to enlighten the public regarding the harmful effects of pollution and its remedial measures so that a cleaner environment may be obtained. History of civilization reveals that water supply and civilization are almost synonymous. Millions of people all over the world particularly in the developing countries are losing their lives every year from water borne disease. The quality of water is of vital concern for mankind since it is directly linked with human welfare.

Some important organic and inorganic water pollutants, their sources as well as their significance are listed below:

Pollutant	Source	Effect/Significance
Cadmium	Industrial discharge, mining waste, metal plating and water pipes.	Replaces zinc biochemically, causes high blood pressure, kidney damage, destruction of testicular tissues and red blood cells.
Manganese	Mining , industrial waste, acid mine drainage, alloy industry, dry cell battery factories and glass industry.	Relatively non toxic to animals and irreversible damage to the central nervous system and brain (ataxia).
Mercury	Mining industrial waste, pesticides and coal (chloral kali industry).	Highly toxic as methyl mercury ($HgCH_3$).Toxic even at a concentration of 0.03 ppm in drinking water. Hg activates sulphur containing enzymes with active – SH group. Affects brain cells and central nervous system.
Copper	Metal plating industry, domestic waste, mining and mineral leaching.	Essential tissue element but toxic to plants and algae at moderate levels. Excess intake is injurious to health.
Cobalt	Magnet making concerns and industrial discharge.	Essential at moderate level but at higher concentration causes coronary failure, thyroid dysfunction due to impaired accumulation of *iodine.*

Pollutant	Source	*Effect/Significance*
Iron	Water flowing through Basaltic rock region and industrial effluents.	Essential for many biological activities. Cause homochromatosis, tension in gastrointestinal tract, shock and liver damage.
Lead	Auto exhaust emission as ethyl lead and industries (glass, paint, mining, plumbing, coal, gasoline etc.).	Toxic, deactivates or destroys sulphur containing proteins and enzymes, damages deoxyribonucleic acid (DNA), ribonucleic acid(RNA), brain and central nervous system function. Inhibits the formation of haemoglobin.

In the present study the migration behaviour (sorption equilibria) of certain pesticides like phosphamidon and dimethoate through pure soil bed has been studied. These studies will be helpful towards assessing movement of these pesticides in soil and controlling soil pollution. It is worthwhile to say a few words about pesticides.

1.3 PESTICIDES

Soil provides us a place to live and is a reservoir of minerals but accelerating growth of world's population and rapid urbanization has put tremendous pressure on it. Insecticides, herbicides and other pesticides aiding agriculture development and health protection are very potent pollutants having long range effects and their indiscriminate use poses a serious danger to the structure and function of the ecosystems. Pesticides while eliminating the beneficial insects may cause ecological imbalance. At the same time, they are phytotoxic and destroy useful plants. They enter the food chain causing toxicity to human beings, animals and other

creatures. Many books (10–15) and reviews (16–18) have been published on this subject as a warning of potential hazards.

Generally, pesticides are classified into two broad categories namely:

i) Inorganic pesticides

ii) Organic pesticides

i) Inorganic Pesticides:

Inorganic pesticides are known to cause several harmful effects. Some of the examples of well known inorganic pesticides are (i) arsenic compounds such as paris green [$(CH_3COO)_2$ $Cu.3Cu(A_sO_2)_2$], basis copper arsenate [$Cu(CuO.\ HASO_4)$] (ii) mercury compounds such as mercuric chloride ($HgCl_2$) (iii) boron compounds such as borax ($Na_2B_4O_7$) and (iv) fluorine compounds such as calcium fluorosilicate ($CaSiF_6.2H_2O$).

ii) Organic Pesticides:

The era of organic pesticides began in 1939 (19) when dichlorodiphenyl trichloroethane (DDT) was discovered. Infact, this compound was synthesized in 1874 by *Zeidler*. Presently thousands of organic compounds have been synthesized and tested for their pesticidal properties. Organic pesticides may be classified as

a) ***Organohalogenated pesticides***: Aldrin, DDT, BHC, endosulphan etc. are some of the organohalogenated pesticides used for crop protection. These pesticides are toxic to insects at very low dose. These compounds tend to accumulate in food chains and are well soluble in organic solvents including fat but are poorly soluble in water.

b) ***Organophosphorus and sulphur containing pesticides:*** These pesticides (dimethoate, metasystox, chloropyrifos, phorate etc.) have a wide range of toxicity, but they are usually non–persistent

and hence poses less threat to the environment than organochlorine.

c) ***Organocarbamate pesticides***: Carbamates belong to a new and comparatively small group of pesticides of growing utility. They bridge the gap between the persistent organochlorine and the short lived but excessively toxic organophosphorus pesticides. This group include, aldicarb, baygon, carbaryl, carbofuran etc. The mode of action of carbamate is similar to that of organophosphorus pesticides.

d) ***Pyrethroids***: The first synthetic pyrethroid appeared in 1973 and many new photostable pyrethroids were synthesized during 1973–1977. These compounds exhibit high activity against insects, low mammalian toxicity, increased stability, effectiveness at very low dosages, rapid action and degradation to innocuous residues. These compounds are more effective as contact pesticides e.g. permethrin, cypermethrin, decamethrin and fanvalerate.

e) ***Miscellaneous pesticides***: Since search and synthesis of new pesticides have always been in consideration and with time more and more pesticides are being listed which do not fall under any of the above categories, such pesticides (e.g. formamidines, amitaz and chlorodimeform) come under this group. These compounds have slow deleterious effect on the health of the crops, soil organisms and human beings.

Pesticides are introduced into the soil for destroying soil-dwelling pests, nematodes and the pathogens of bacterial and fungal disease. The property of pesticides to withstand the decomposing action of physical, chemical and biological processes characterize their persistence. The persistence of pesticides in the soil also depends upon the dose, formulation (powder, liquid etc.), the type of soil, its moisture content and temperature. Pesticides as a rule are more persistent in soils with a high content of organic matter. Pesticides introduced into the soil, lose a

part of their being and are adsorbed by the soil colloids. The degree of adsorption of most pesticides largely depends on the humus content in the soil. It also depends upon the pH, pK and hydrolytic acidity of the soil.

1.4 CHROMATOGRAPHY

It is a physical method of separation in which the components to be separated are distributed between two phases, namely (i) stationary phase, which can be a solid or a liquid support on a solid and (ii) mobile phase (a gas or a liquid) which flows continuously through the stationary phase. The separation of individual components results primarily due to differences in their affinity for the stationary and mobile phases.

Chromatography has been defined by *Cassidy* (20) as "*a separation process applicable to essentially molecular mixtures which relies on distribution of the mixture between an essentially two dimensional or thin phase and one or more bulk phases which are brought into contact in a differential countercurrent manner*". The word "*Chromatography*" was first used by Russian botanist *Michael Tswett* (1906) to describe separation of plant pigments, which was effected by passing an extract of green leaves through a column packed with fine grains of calcium carbonate. Since the separation results into formation of a series of coloured zones, the name "*Chromatography*", derived from the Greek words *chromatus* and *graphein*, meaning "*colour*" and "*to write*" was used by him. After the initial work of Tswett, a wide variety of independent techniques that have little or nothing to do with colour have come to be called chromatography. From its infancy, chromatography has grown in significance and popularity to become a leading technique of analysis. The salient features of common chromatographic techniques are summarized in **Table 1.1.**

Since the work summarized in this thesis is based mainly on the use of thin-layer chromatography as a separation technique, it is therefore worthwhile to provide the important aspects of this technique. The following pages are devoted to outline the development and current state-

Table 1.1

Salient features of common chromatographic techniques

Technique	Principle	Sample	Remarks
Gas chromatography (GC)	It involves the partitioning of gaseous solutes between inert gas mobile phase and a stationary liquid or solid phase.	Solid, liquid or gaseous volatile organics.	Introduced in 1950's, useful for analysis of organic and inorganic compounds but unsuitable for analyzing proteins and polymers. Sensitivity, ng/L.
Liquid chromatography/ High performance liquid chromatography (LC/ HPLC)	Separation is controlled by the differential distribution of solutes between the mobile and stationary phases. The solutes are resolved by their differential rates of elution.	Liquid volatile and nonvolatile organic, inorganic and biological compounds, polymers, chiral compounds, thermally labile compounds, small ions and macromolecules.	A leading analytical technique for components not suitable for volatilization. Developed in the late 1960's. Sensitivity µg/L.
Capillary electrophoresis (CE)	The charged species are separated on the basis of their different migration rates under the influence of an electric field.	Liquid polar and non–polar compounds, some elements non–ionic and ionic organics, inorganic anions and macromolecules and compounds.	Useful in separation and identification of polar and non–polar compounds, as well as macromolecules (proteins, oligonucleotides. Sensitivity, µg/L.

Technique	Principle	Sample	Remarks
Supercritical fluid chromatography (SFC)	It is the hybrid of HPLC and GLC, permitting the separation of compounds of very high molecular weight. The mobile phase is cooled to retain the molecules in a liquid state for pumping and then heated in an oven until its critical temperature.	Solid, liquid or gaseous, thermally labile and non–volatile analytes.	Separation of a wide range of volatile and non–volatile compounds (organic, organometallic, biological, polymer, chiral and thermally labile). Sensitivity, ng/L.
Ion chromatography (IC)	It is based upon ion exchange chromatographic separation with simultaneous detection of ionic species.	Inorganic anions or cations, organic acids, amines, amino acids, carbohydrates and nucleic acids.	Provides separations of organic and inorganic ions and ionisable species. Sensitivity, µg/L.
Planar layer chromatography (PLC)	Separation is based on the differential distribution of solutes between a planar stationary phase and a liquid mobile phase. Application is limited to the analysis of non–volatile compounds.	Same as for liquid chromatography.	Useful for separation of almost all compounds (organic, inorganic, biological, polymers, chiral etc.). Common applications include environmental, pharmaceutical, biomedical and food analysis. Sensitivity, µg/L.

of-art of thin layer chromatography (TLC) as used to the analysis of inorganics, amines and phenols.

1.5 THIN–LAYER CHROMATOGRAPHY

TLC is a subdivision of liquid chromatography in which the mobile phase (a liquid) migrates through the stationary phase (a thin layer of porous sorbent on a planar inert surface) by capillary action. It is a rapid, simple, versatile, reasonably sensitive and inexpensive analytical tool, which is applicable for both qualitative and quantitative analyses of several compounds.

1.6 HISTORY OF TLC

History of TLC has been reviewed by *Stahl* (21), *Kirchner* (22,23) and *Pelick* et al. (24). In fact, the beginning of TLC may be attributed to *Beyerinck* who reported the separation of sulphuric and hydrochloric acids in the form of rings on thin layer of gelatin (25). Following the same techniques, *Wijsman* separated enzymes from malt diastase (26). In 1938, *Izmailov* and *Schraiber* separated certain medicinal compounds on binder free horizontal thin layer of alumina spread over a glass plate (27). As the development was carried out by placing solvent drops on the glass plate-containing sample and adsorbent, their method was called "*drop chromatography*". However, this method could not catch the eyes of scientists until two American chemists, *Meinhard* and *Hall* used a mixture of aluminium oxide (adsorbent) and celite (binder) as a layer on a microscopic slide to separate inorganic ions (28). They called this technique as "*surface chromatogaphy*" and this was the first application of layer chromatography in the separation of inorganics. Since 1958, when *Stahl* introduced the term "*thin layer chromatography*" and standardized procedures, materials and nomenclature (29,30), the effectiveness of this technique for separation was realized.

A major breakthrough in the field of TLC came in the early 1960's with the availability of precoated plates (31). It had recently been realized that modern high performance thin layer chromatography (HPTLC) initiated in 1975, rivals high pressure liquid chromatography (HPLC) and gas chromatography (GC) in its ability to resolve complex mixtures and to provide analyte quantification.

1.7 COMPARISON OF TLC, HPLC AND HPTLC

According to recent literature (32), TLC has distinct advantages over HPLC, e.g. greater detection possibilities, more rapid throughput, use of disposable plates, easier sample preparation, low solvent consumption and lower operational cost. The poorer separation efficiency and the influence of environmental conditions on the reproducibility of R_F value have, however, been major disadvantages of TLC compared with HPLC and GC.

The above-mentioned advantages of HPTLC/TLC have made it the premier method for assessing atmospheric, aquatic and residual pollution. It has been successfully applied for the analysis of wastewater for total heavy metal contents (33), characterization of hazardous wastes (34), identification of metals in sludge sample (35), and quantification of toxic metals in industrial sewage (36). Compared to conventional TLC, HPTLC provides faster separations, reduced zone diffusion, better separation efficiency and higher sensitivity. However, HPTLC is not in common use because of high capital investment, procedural difficulties and instrumental complications. A comparison of TLC and HPTLC characteristics is summarized in **Table1.2**.

Table 1.2

Comparision of TLC and HPTLC

Parameter	TLC	HPTLC
Plate size	20 x 20cm	10 x 20 or 10x 10cm
Average particle size	20μm	5μm
Adsorbent layer thickness	100–250μm	200μm
Plate height	30μm	12μm
Sample volume	1–5μm	0.1–0.2μL
Solvent migration distance	10–15cm	3–6cm
Separation time	30–200min	3–20min
Samples per plate	10	18 or 36
Detection limits		
Absorption	1–5ng	0.1–0.5ng
Fluorescence	0.05–0.1ng	0.005–0.01ng
Diameter of separated spots	6–15mm	2–6mm

HPTLC provides faster separation, reduced zone diffusion, better separation efficiency and higher sensitivity.

1.8 TLC METHODOLOGY

The complete process of TLC is summarized in **Fig1.1**. Solute identification in TLC is based on R_F, known as retardation factor, which is calculated as

$$R_F = \frac{Distance\ travelled\ by\ the\ compound\ from\ the\ origin}{Distance\ travelled\ by\ the\ mobile\ phase\ from\ the\ origin}$$

The R_F values in TLC are dependent upon many variables (nature of the sorbent, layer thickness, layer-activation temperature, chamber saturation, nature of mobile phase, pH of the medium, development technique used, room temperature, sample size and relative humidity etc.) which must be regulated carefully during the preparation and evaluation of the chromatogram to obtain reproducible results. R_F values vary between 0.0 (solute remaining at the point of application) and 0.999 (solute migrates up to the solvent front) and have no unit. The differential migration results because of varying degrees of affinity of components in a mixture, for the stationary and mobile phases.

1.9 PRINCIPLE AND TECHNIQUES

In TLC, separation of components from a mixture is achieved by optimizing the experimental conditions. The desired separation can be achieved by proper selection of adsorbent (stationary phase) and solvent (mobile phase) system.

Nature of Phase Interactions

Some of the important physical and chemical characteristics that determine the degree of interactions of mobile phase-solute, sorbent–solute and mobile phase- sorbent are given in the following paragraph.

a) *Intramolecular Forces*: These forces hold neutral molecules together in the liquid or solid state. These physical forces are characterized by low equilibrium and results in good chromatographic separation.

b) *Inductive forces*: These forces exist when a chemical bond has a permanent electrical field associated with it (e.g. C-Cl, C-NO_2 groups). Under influence of this field, the electrons of an adjacent atom, group or molecule are polarized so as to give an induced dipole moment. This is a major contributing factor in the total adsorptive energy on alumina.

c) *Hydrogen Bonding*: It makes a strong contribution in adsorption energies between solute or solvents having a proton donor group and a nucleophilic polar surface such as that of alumina or silica gel.

d) *Charge-Transfers*: Charge-transfer between components of the mobile phase and the sorbent may also take place to form a complex of the type S^+A^- (where S= solvent or solute, and A= surface active center of sorbent). This phenomenon is prominent in ion–exchange chromatography.

e) *Covalent Bonds*: Covalent bond can be formed between solute and/or the mobile phase and the sorbent. These are strong forces and result in poor chromatographic separation.

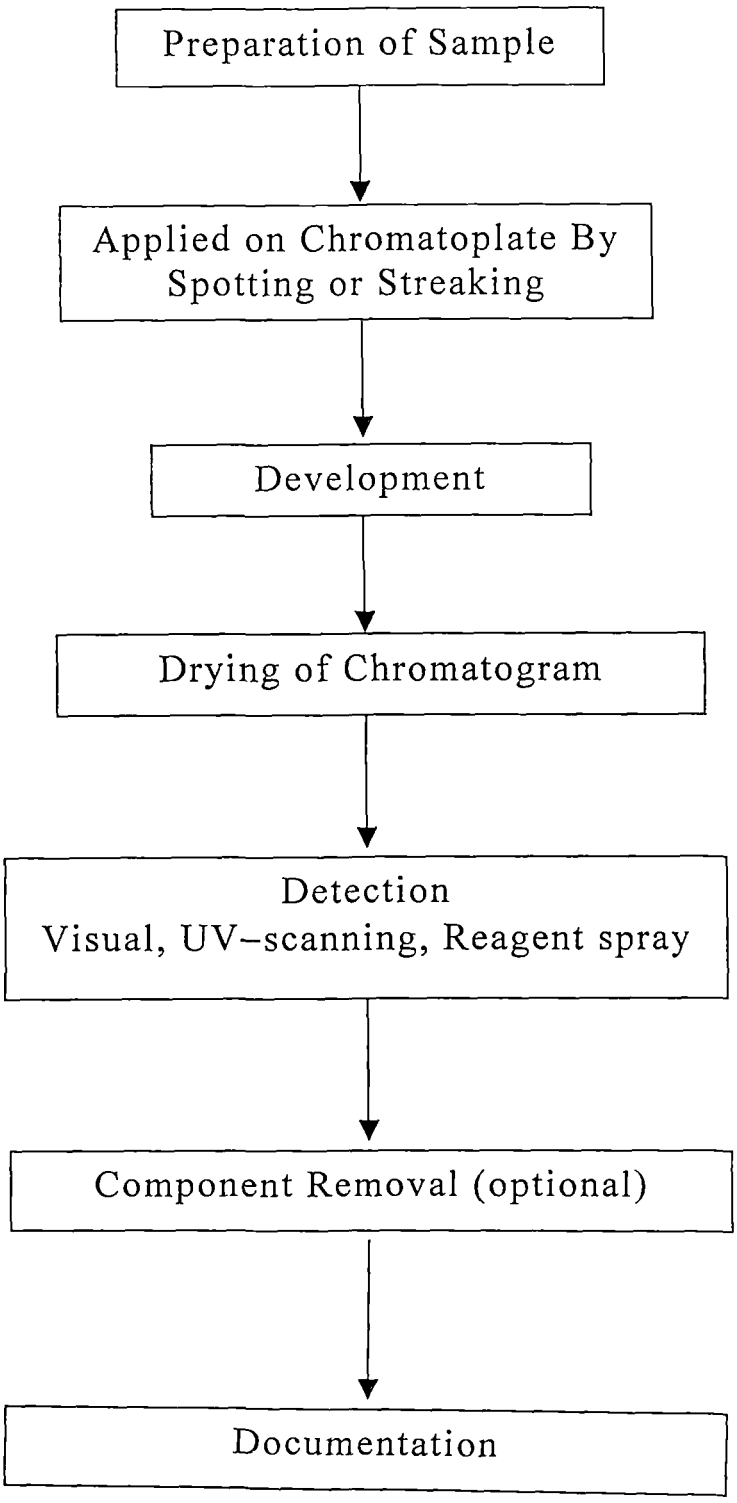

Figure 1.1 The process of thin–layer chromatography

1.10 CHROMATOGRAPHIC SYSTEMS

The optimum conditions for separation in TLC are yielded through mutual harmonization of stationary and mobile phases.

Stationary Phase (Adsorbent)

A large number of sorbents are available which can be used in TLC. In fact no perfect adsorbent has been found, some have great retaining power whereas others hold the adsorbate loosely. The adsorbing power of a substance varies greatly according to its particle size, method of preparation, activation process etc.

A good adsorbent should possess the following characteristics:

1. The adsorbent must not chemically interact with the materials being investigated.
2. The colour of the adsorbent should be such that it does not interfere with the chromatogram, preferably it should be colourless.
3. The adsorbent should be insoluble in the solvent to be used.
4. It should be non-catalytic i.e. should not catalyze the decomposition of the substances.
5. The physical and chemical properties of one adsorbent should not change under the experimental conditions.

Some commonly used sorbents are silica gel, alumina, cellulose and kieselguhr (diatomaceous earth).

Silica gel is the most frequently used layer material. It is slightly acidic in nature. At the surface of silica gel the free valencies of the oxygen are connected either with hydrogen of silanol (Si-OH) groups **(Fig1.2)** or with another silicon atom of siloxane (Si-O-Si) groups. The silanol groups represent adsorptive active surface centers that are capable to interact with solute molecules. The ability of the silanol groups to react chemically with appropriate reagents is used for controlled surface

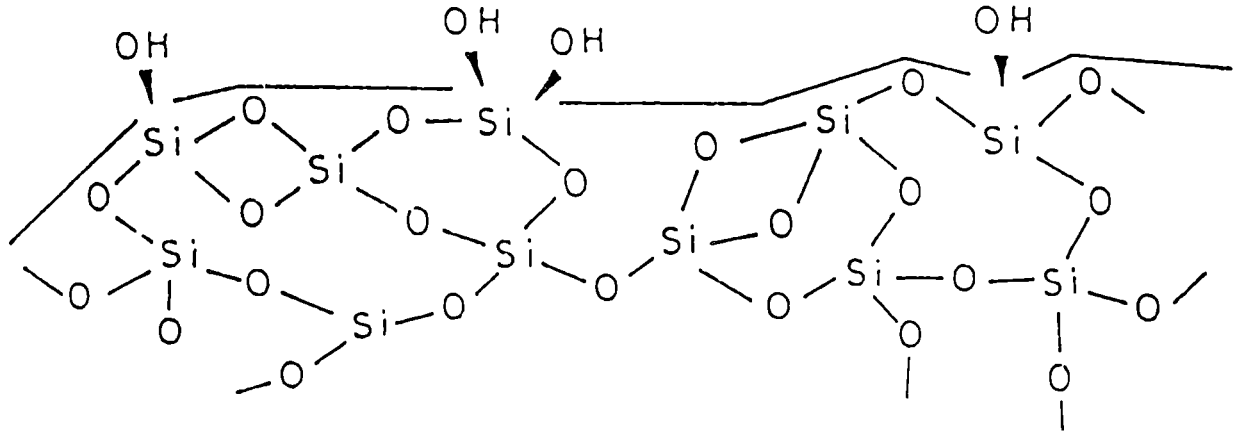

Figure 1.2 Structure of silica gel

modifications. Hence, silica gel is considered as the most favoured layer material in chromatography.

Alumina (aluminium oxide, $Al_2 0_3$) is also widely used as a sorbent. It is more reactive than silica gel, but for a given layer thickness it will not separate quantities of material as large as can be separated on silica layer. Adsorption is the separation mechanism in both silica gel and alumina. The surface-active centers of alumina are hydroxyl groups and oxide ions. In aqueous suspensions, alumina surface is capable to bind with metal ions, anions or metal complexes. Chromatographic properties of alumina are influenced by the adjustment of pH value. Three ranges of pH values have proved suitable for aluminas i.e. pH values of 9-10 (basic aluminas), pH values 7-8 (neutral aluminas) and pH values 4-4.5 (acidic aluminas).

Cellulose can be used as a sorbent in TLC when it is convenient to perform a given paper chromatographic separation by TLC in order to decrease the time required for the separation or to achieve increased detection sensitivity. In general, two types of cellulose such as (i) native cellulose and (ii) microcrystalline cellulose are in use. Native cellulose is fibrous and has a degree of polymerization of 400-500 glucose units whereas microcrystalline cellulose consists of approximately 40-200 of glucose units. Cellulose are mainly used in partition TLC for the separation of relatively polar compounds.

Kieselguhr is chemically neutral sorbent which consists of $Si0_2$ (90%) and about 10% of Al_2O_3, Fe_2O_3, MgO, Na_2O, K_2O, CaO and $Ti0_2$ in different proportions. Kieselguhr has very low surface activity and hence used mostly to separate herbicides and aflatoxins in a partition chromatographic mode.

The various types of sorbent layers presently in use may broadly be clubbed together as follows:

a) *Non-surface Modified Layers or Untreated Sorbents*

The sorbent phases used in the non-modified form include silica gel G, silica gel H, silica gel LS, acidic and neutral alumina, cellulose, polyacrylonitrile etc.

b) *Impregnated or Treated Sorbents*

To broaden the range of selectivity of above mentioned layer materials in (a), several workers attempted to use these materials after impregnation to a definite degree with buffers, metal ions, chelating agents or high molecular weight organic liquids. Some of the impregnated adsorbents used as layer material include: silica gel impregnated with aqueous inorganic salt solutions; acid treated silica gel; silica gel impregnated with chlorobenzene, high molecular weight amines, tributylamine, tributyl phosphate, chelating agents such as EDTA, ammonium rhodenate or mixture of alizarin red S and aliquat 336; silica gel impregnated with surfactants, mono- (2-ethylhexyl) acid phosphate or p-toluidine and silufol impregnated with 5% paraffin oil.

c) *Chemically Modified or Bonded Sorbents*

The impregnated layers as mentioned in (b) suffer from the limitations such as (i) the impregnants are eluted to some extent by the mobile phases used and (ii) the stripping of liquid stationary phase from the support by incompatible mobile phases.

To overcome these problems, chemically bonded layer materials of similar properties were developed for safer use as stationary phase. Some of the examples are: lipophillic C_{18} -bonded silica gel phases, aminopropyl silica gel (NH_2), octadecyl silica gel (C_{18}) and surface-modified cellulose like ECTEOLA (a reaction product of epichlorohydrin triethanolamine and alkali celluose).

d) *Inorganic Ion – Exchangers*

Apart from silica and alumina, other inorganic ion-exchangers have also found use in TLC. Besides many others, the use of stannic silicate, zirconium phosphoantimonate, zinc ferrocyanide, stannic sulfosalicylate, binder free zirconium (iv) antimonate and hydrous antimony (v) oxide as layer material has also been reported.

e) *Mixed Sorbents*

Mixed layers (impregnated and non-impregnated) have been used by several workers for achieving enhanced resolution of components. Mixed layers are usually of medium activity as compared to the separated phases. The addition of kieselguhr in silica generally reduces the activity of silica, resulting in a new sorbent layer with altered activity that is capable of providing peculiar separations, not possible on separated phases. The binary layers that have been used include silica gel- microcrystalline cellulose (MCC) containing NH_4NO_3, silica gel G-MCC, silica gel- inorganic ion-exchange gels or Zr (IV) antimonate, MCC-modified silica gel H, silica gel-alumina or anitmonic acid and kieselguhr-cellulose.

f) *Miscellaneous Sorbents*

The layer materials that are less familiar or introduced recently in TLC have been put under this category. These materials include silufol; silufol UV 254; silufol with a layer of silica gel; soil; soil-flyash, soil treated with neutral, alkaline and saline solutions; soil mixed with silica gel and kieselguhr etc.

A part of the work presented in this thesis is related to the analysis of pesticides and heavy metal cations on layers prepared from soil containing adsorbents. It is, therefore, necessary to provide a brief description of soil. The following paragraphs are devoted to encapsulate available information about soil, which has been used as stationary phase during present study.

1.11 SOIL

The term soil is derived from the Latin word "*solum*" which means floor. The soil is the key component of terrestrial ecosystem and is essential for the growth of plants as well as for recycling of dead biomass. It is a complex heterogeneous medium consisting of minerals, organic solids and aqueous as well as gaseous components. The minerals present are usually rock fragments and secondary minerals (phyllo-silicates or clay minerals), oxides of Fe, Al and Mn and sometimes carbonates (usually $CaCO_3$). The term 'oxides' includes all forms of oxides including hydrous oxides and oxyhydroxides. The organic matter comprises living organisms (mesofauna and microorganisms), dead plant material (litter) and colloidal humus formed by the action of microorganisms on plant litter.

The soil is a dynamic system, subject to short-term fluctuations, such as variations in moisture status, pH and redox conditions and also undergoing gradual alterations in response to changes in management and environmental factors. These changes in soil properties affect the form and bioavailability of metals, and need to be considered in decisions on the management of polluted soils or the use of soils for disposal of waste material.

Composition of Soil

From the physical stand point, soil is composed of solid, liquid and gaseous phases in varying proportions. In an ideal soil surface, the various observed components (by volume) include: (a) mineral matter–40%, (b) organic matter–10%, (c) soil matter–25% and (d) soil air-25%.

The proportion of above mentioned components may vary from time to time and from place to place. The inorganic (mineral) material in soil is relatively fixed but the organic material may vary depending upon the location of the collection of the soil sample. The volumes of water and air in soils are controlled by several factors such as particle size, porosity, humidity and temperature of the soil environment.

a) *Mineral Matters (Clay Minerals)*

Clay minerals are products of rock weathering and have marked effects on both the physical and chemical properties of the soils. Their contribution to soil chemical properties results from their comparatively large surface area and permanent surface negative charge. The clay minerals consist of continuous two dimensional tetrahedral sheets of composition T_2O_5 where T is tetrahedral cation, usually Si^{4+} or Al^{3+}.

Some important and frequently occurring clay minerals are:(i) kaolinite,(ii) montmorillonite,(iii) illite,(iv) chlorite,(v) amorphous clays

i) *Kaolinite*

The name *'kaolinite'* was first suggested by "*Johnson* and *Blake*" in 1867. The structure of kaolinite is based on single tetrahedral sheet topped by a slightly distorted gibbsite sheet, both being joined by oxygen bonding through condensation and splitting of water between adjoining hydroxyl groups in vertical position. Water can not enter in these lattices which explains the comparatively low moisture content as well as low swelling of these clays. The size of unit cell is 7.2 A^0. The structural formula of kaolinite is $(OH)_8Al_4Si_4O_{10}$. The cation exchange capacity of a mineral is very low and varies from 3 to 15 meq$100g^{-1}$ clay. Similarly, plasticity, cohesion and shrinkage properties of kaolinite are low.

ii) *Montmorillonite*

This mineral was first studied by *LeChatellier* and has been assigned the formula $(OH)_4Al_4Si_8O_{20}$. The structure of montmorillonite is composed of simple octahedral sheet of alumina enclosed between two silica sheets. It possess high cation exchange capacity which varies from 80 to120 meq $100g^{-1}$ clay.

iii) *Illite*

The name, "*illite*" was proposed by *Grim* in 1937 for the mica like minerals with a $10A^0$ C-axis which shows no expansion in the

presence of water. The size of unit cell is $10A^0$. Isomorphic substitution is possible within the lattice resulting in a wide variation in composition. Because of its resemblance with mica, it is considered to be a member of mica group. The cation exchange capacity of illites varies from 20 to 40 meq $100g^{-1}$ clay.

All these three types of clays strongly adsorb organic cations (37-39).

iv) *Chlorite*

The structure of *chlorite* was given by *Donahue* et al. It is often called 2:2 type clay. The crystal unit consists of two silica tetrahedral sheets and two magnesium octahedral sheets (2:2). Chlorites do not swell when wet with water. The cation exchange capacity of chlorite is very low and varies from 10 to 14 meq $100g^{-1}$ clay.

v) *Amorphous Clay*

Amorphous clays are mixtures of silica and alumina that have not formed from well oriented crystals. These clays are common in soils forming from volcanic ash. Amorphous clays are not well characterized but to exist in many soils in varying amounts. Their properties are often quite unusual, such as having high anion or cation exchange capacities that vary from 50 to 150 mol Kg^{-1} clay.

b) *Soil Organic Matter (SOM)*

Organic matter is an active and reactive portion of the soil. It may be defined as organic residue left behind after microbial decomposition of plant and animal remains, frequently dark coloured and possessing certain physical and chemical properties. The organic matter content of the soil is generally found in smaller quantities that is 1-5% by weight in a top soil, and decreases with depth. Organic matter and clay minerals are the two important causes which are responsible for ion exchange property (40-41). Colloidal soil organic matter has a major influence on the chemical properties of soils, and can be divided into *'non-humic'* and

'humic' substances. The non-humic substances comprise unaltered biochemicals such as amino acids, carbohydrates, organic acids, fats and waxes that have not changed from the form in which they were synthesized by living organisms. Humic substances are a series of acidic, yellow to black coloured polyelectrolytes of moderately high molecular weight. They are formed by secondary synthesis reactions involving micro-organisms and have characteristics which are dissimilar to any compounds in living organisms (42). They have a wide variety of functional groups, including carboxyl, phenolic hydroxyl, carbonyl, ester and possibly quinone and methoxy groups (43,44). Soil humus also contains some biochemicals bound to the humic polymers. Traditionally, humus has been separated in the laboratory into three fractions:

i) humic, which is insoluble in alkali and acid

ii) humic acid, which is soluble in alkali and insoluble in acid

iii) fulvic acid, which is soluble in both acid and alkali

Methods used to determine the organic matter content of soils include either the percentage loss in weight after ignition in a furnace at 375^{0}C for 16h or the oxidation of C by acidic dichromate followed by the titration of excess dichromate (45).

c) *Soil Water*

In soil, water is supplied to plants through the roots. It "*lubricates*" the soil allowing root penetration which is necessary for microbial mobility. Hence, water has perhaps the greatest influence on the growth and yield of a crop. It is needed in much larger quantity than that of any other substance that contributes to growth and yield. The soil water content varies with soil texture and ranges from 5.1 to 11.9%.

d) *Soil Air*

Percentage composition of atmospheric air (by volume) may be approximately taken as N_2 = 79.0, O_2=20.97, CO_2=0.03. The inert gases are included in nitrogen percentage. The composition of soil air varies

according to the nature of soil as well as with the climatic conditions e.g. composition of CO_2 which increases after rainfall, probably because of increased nitrification and decomposition of organic matter. The composition of soil air is characteristically different under anaerobic condition. For example, the percentage of CO_2 increases in swamp soil. CO_2 production being a product of biological activity is dependent on temperature, organic matter, moisture and the condition of soil.

Physico-Chemical Properties of Soil

The knowledge of physico-chemical properties of soil is important in understanding soil behaviour. It includes the distribution of particle size such as sand, silt and clay, soil pH, electrical conductivity and cation exchange capacity etc.

Mechanical Composition

The first information required about a soil is its mechanical composition. The mechanical composition or texture means the particle of various sizes such as gravel, sand, silt and clay in soils. The particle size distribution of a soil influences its chemical, physical and biological properties. Particle size distribution in soil influences the water holding capacity. It also influences the strength and compressibility of soils. The success of any mechanical analysis primarily depends upon the preparation of sample to ensure complete dispersion of all aggregates into their individual particles without breaking up the particles, and secondary upon the accurate fractionation of the sample into various fractions.

Soil pH

The pH of soil is an approximate measure of an active fraction of the hydrogen ions present in the soil phase. The pH of a soil applies to the H^+ (ions) concentration in the solution present in soil pores which is in dynamic equilibrium with the predominantly negatively charged surfaces of the soil particles. Hydrogen ions are strongly attracted to the surface negative charges, and they have the power to replace most other cations. Soil pH is affected by the changes in redox potential which occur in soils

that become water logged periodically. Soil have several mechanisms which serve to buffer pH to varying extents, including hydroxyl aluminium ions, CO_2, carbonates and cation exchange reactions (46). However, even with these buffering mechanisms, soil pH differs significantly due to localised variations within the soil.

Electrical Conductivity

It gives an idea about the total soluble salts present in soil. Soil salinity scale is based on electrical conductance of the extract. For saline soil, the soil content is more than 0.3% or E.C.> 4.0 $ohm^{-1}cm^{-1}$. The salinity is due to the presence of NiCl and Na_2SO_4 as soluble salts in soil.

Cation Exchange

Most heavy metals (with certain exceptions, including the metalloids As, Sb and Se and the metals Mo and V) exist mainly as cations in the soil solution, and their adsorption therefore depends on the density of negative charges on the surfaces of the soil colloids. In order to maintain electroneutrality, the surface negative charge is balanced by an equal quantity of cations. Ion exchange refers to the exchange between the counter–ions balancing the surface charge on the colloids and the ions in the soil solution (47) It has the following characteristics: Ion-exchange is reversible, diffusion controlled and stoichiometric process which involves the preference for one ion over another by the adsorbent (48). The cation exchange capacity (CEC) of mineral soils can range from a few to 60 C $mols_c$/kg but in organic soils it may exceed 200 C$mols_c$/kg (49).

Soil Thin-Layer Chromatography

Soil thin layer chromatography was first successfully utilized in 1968 by *Helling* and *Turner* for the detection of pesticides movement using different types of soil as static phase. The movement of substances or their separations in TLC can be affected by altering the conditions of the static phase (soil) of diverse nature, developer and applied substances. Thus, soil TLC provides a very fascinating field of research which can be

utilized for investigating problems in various applied and non-applied fields.

Thin layers of soil are prepared by spreading an uniform film (0.15-2.0 mm) of soil slurry over a glass plate with the help of an applicator and allowing it to dry at room temperature (30°C). The organic or inorganic compound, under investigation is spotted above 3 cm from the bottom of the plate so that the mobile phase level in the closed jar may always remain about 1cm below the spots. Thin layer plates are developed to a distance of 10 cm starting from the point of spotted compound. After the development , the plates are removed from jar, dried and the compounds or ions are detected by spraying suitable chromogenic reagents as detectors.

The movement of the compound through the soil layer is measured in terms of R_F values which can be calculated as

$$R_F = \frac{\textit{Distance travelled by solute from the point of application (i.e. origin)}}{\textit{Distance travelled by solvent from the line of sample application (i.e. origin)}}$$

1.12 MOBILE PHASE (solvent system)

In liquid chromatography including TLC, the mobile phase exerts a decisive influence on the separation. Various optimization schemes (Windows diagram, overlapping resolution maps, simplex method and PRISMA model) have been proposed for normal–phase and reversed-phase TLC. Mobile phase should be as simple as possible and be prepared from the purest grade of the solvent available. Mixtures composed of more than three or four components should be avoided because of problems associated with reproducible preparation. Care must be taken to mix the constituents thoroughly before use. With a particular sorbent layer, the separation possibility of a complex mixture is greatly improved by the proper selection of mobile phase. The mixture of organic solvents containing some aqueous acid, base or a buffer are, in general, well suited for the separation of ionic species whereas anhydrous organic solvents and

water containing mobile phases are more useful for separating non–ionic species. The following mobile phases have been used as developers:

a) ***Organic solvents***: The single component mobile phase including acetone, acetonitrile, benzene, carbontetrachloride, chloroform, dioxane, ethanol, ethylacetate, methanol, o-xylene, petroleum ether, toluence, n-octanol, n-nonane, cyclohexane and binary/ternary mixtures of alcohols, amines, ketones, phenols and haloalkanes have been used.

b) ***Inorganic solvents***: Being non–toxic and non–volatile, solvent systems of this group have been widely used in TLC of inorganics and organometallics. This group includes the solution of mineral acids, alkalies and inorganic salts prepared in double distilled water or water–methanol mixture.

c) ***Mixed solvents***: Mixtures of two or more different solvents, most of which have either a base (NaOH, NH_4OH and amine) or an acid (mineral or carboxylic) as a component, are used to develop the TLC plate.

d) ***Surfactant–mediated solvents***: Solutions of surfactants (SDS, CTAB or Triton X–100) have also been used but to lesser extent as mobile phase in TLC.

The traditional mobile phase systems as mentioned above (a–c) have been widely used in TLC analysis of organic as well as inorganic substances. However, the use of surfactant–mediated mobile phase systems in TLC is of recent origin. These systems, though, have find extensive use in HPTLC, their use in TLC has been limited.

Since the major portion of the work presented in this thesis is related to the use of the surfactant containing mobile phases, it is worthwhile to mention the salient features regarding the behaviour of surfactants in aqueous media as well as their utility in chromatography.

Surfactant–Mediated Systems

These systems contain surfactant as one of the components of the mobile phase. Surfactants in the aqueous mobile phase can be used in the following ways:

(a) As *monomer* surfactants where the concentration of surfactant in aqueous mobile phase is restricted to well below the critical micelle concentration (CMC) of the surfactant. These mobile phases are most suited to separate ionic species by ion–pair chromatography (IPC). In this technique, a small concentration of ion–pairing reagent, which has an opposite charge to the ionic solutes (i.e. cationic surfactant for anionic solutes and anionic surfactant for cationic solutes) is added to the aqueous mobile phase and its concentration is kept low in order to avoid the formation of micelles.

(b) As surfactant *micelles* where the surfactant concentration is kept well above its critical micelle concentration (CMC) value. In such cases, the mobile phase is composed of surfactant molecules in the form of monomers and aggregates (or micelles). These mobile phases are very useful for simultaneous separation of ionic and non–ionic compounds by micellar liquid chromatography (MLC).

(c) As *microemulsion* where surfactant in the presence of water, an oil (hydrocarbon) and co–surfactant (i.e. medium chain length amine or alcohol) is used as transparent solution.

Surfactants are long chain amphiphilic organic or organometallic molecules containing a highly polar (hydrophilic or lipophobic) or ionic "*head group*" attached to a non-polar (hydrophobic or lipophilic) hydrocarbon "*tail*" of varying chain length. The "head group" is either cationic (e.g. ammonium or pyridinium ion), anionic (e.g. hydroxy-compounds) or zwitterionic (e.g. amino oxide, carboxylate or sulphonate betain) and the hydrocarbon tail which may contain at least 8 carbon atoms. Since surfactant molecules are surface-active i.e. capable to lower

the surface tension of water when dissolved in it, these are sometimes called *surface active agents or detergents.* Owing to the presence of both non-polar (hydrophobic) and polar (hydrophilic) groups, these substances have also been referred to as *amphipathic, tensioactive, tenside, heteropolar* or *polar-nonpolar* substances. The dual character (polar-nonpolar nature) of surfactant molecules is responsible for their unique properties in solution, which render possible applications in dispersion, emulsification, wetting, catalysis, organic synthesis, reaction kinetics and separation technology. The factor responsible for desired surface activity is the balance between hydrophobic and hydrophilic characteristics of these molecules. Depending upon the nature of the hydrophilic group, surfactant can be classified as anionic [$R\text{-}X^-M^+$];catioic [$R\text{-}N^+ (CH_3)_3 X^-$]; zwitterionic [$R\text{-}(CH_3)_2 N^+ CH_2 X^-$] and non–ionic [$R(OCH_2CH_2)]_m OH$ where R is a long aliphatic hydrocarbon chain, M^+ is a metal ion, X^- is a halogen, CO_2 or SO_4^{2-} and m is an integar. A list of some common surfactants is provided in **Table1.3**.

Table 1.3

Typical surfactants and their CMCs, aggregation numbers, kraft point values[a]

Surfactant	CMC(M)	Aggregation number	Kraft point (°C)
Aqueous (normal) anionic			
Sodium dodecyl sulphate (SDS $CH_3(CH_2)_{11}OSO_3^-Na^+$	8.1×10^{-3}	62	9
Potassium perfluoroheptanoate, $C_7F_{15}COO^-K^+$	3.0×10^{-2}	c	25.6
Sodium polyoxyethylene (12) dodecyl ether, $CH_3(CH_2)_{11}(OCH_2CH_{12})_{12}OSO_3^-Na^+$(SDS12EO)	2.0×10^{-4}	81	<0
Cationic			
Cetylpyridinium chloride,[d] $C_{16}H_{33}N^+C_5H_5Cl^-$	1.2×10^{-4}	95	c
Cetyl trimethyl ammonium bromide (CTAB), $CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$	9.0×10^{-4}	78	23
Non–ionic			
Polyoxyethylene (6) dodecanol, $CH_3(CH_2)_{11}(OCH_2CH_2)_6OH$	9.0×10^{-5}	400	c
Polyoxyethylene (23) dodecanol (Brij–35), $CH_3(CH_2)_{11}(OCH_2CH_2)_{23}OH$	1.0×10^{-4}	40	c
Zwitterionic			
N–dodecyl–N, N–dimethylammonium–3–protance–1–sulphonic acid (SB–12), $CH_3(CH_2)_{11}N^+(CH_3)_2(CH_2)_3SO_3$	3.0 x 10–3	55	<0
N, N–dimethyl–N–(carboxymethyl) octylammonium salt, $C_8H_{17}N^+(CH_3)_2CH_2COO^-$(octylbetaine)	25×10^{-2}	24	<0
Non–aqueous (reversed)			
Bis(2–ethylhexyl) sodium sulphosucccinate (AOT),[e] $NaO_3SCH(CH_2COOC_8H_{17})COOC_8H_{17}$	6.0×10^{-4}	c	c

[a] *values for aqueous solution at 25°C*

[b] *Temperature at which the solubility of an ionic surfactant is equal to the CMC.*

[c] *Not available or not defined*

[d] *in 0.175 M NaCl.*

[e] *In hexane.*

1.13 MICELLES

Surfactant (or amphiphilic) molecules comprising of hydrophobic and hydrophilic moities tend to exhibit a considerable degree of self organization when dissolved in aqueous solutions. Above a certain concentration level, termed as critical micelle concentration (CMC), the surfactant molecules in solutions (water or organic solvents) aggregate to form micelles. The process of micelle formation is called "micellization". Micelles do not exist at all concentrations and temperatures. There is a very small concentration range below which aggregation to micelles is absent and above which association leads to micelle formation. This narrow concentration range during which micelle formation occurs is called the CMC. At low concentration (i.e. below CMC), the surfactant is dispersed in the aqueous media at the molecular level as a monomer. The average number of monomers per micelle is called the aggregation number (N). At 25 ^{0}C and 1 atm; the CMC is typically less than 20 mM, with each micelle-consisting of 40-140 monomers.

Regardless of the structure of the hydrophilic moiety (cationic, anionic, non–ionic or zwitterionic), it is generally accepted that hydrophobic interactions are the main driving force for micelle formation in aqueous media. A conventional model of micelle is that proposed by *Hartley* **(Fig.1.3)** which is very useful for visualization of a micelle. Micellar structure is also affected by experimental parameters such as temperature, pressure, pH, ionic strength and the presence of additives in the surfactant solution.

There are mainly two types of micelles (a) normal micelles and (b) reverse micelles.

a) ***Normal Micelles:*** The molecular organization of surfactant molecules in aqueous solutions resulted in the formation of normal micelles. Above CMC, the surfactant molecules are self aggregated in such a manner that the hydrophobic moities (i.e. hydrocarbon tails) are oriented inward forming a non-polar core and hydrophilic

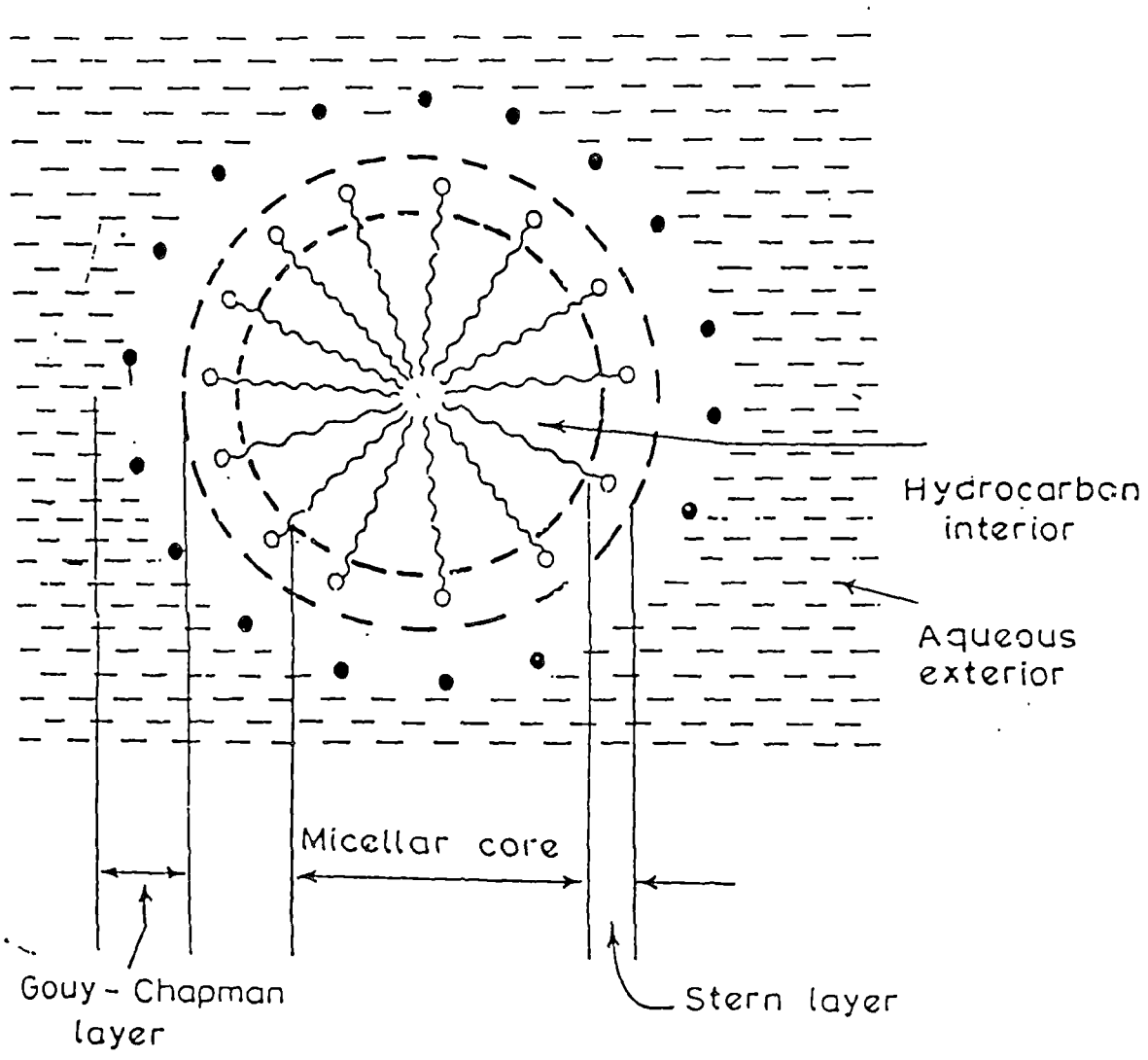

Figure 1.3 Hartley model of a spherical micelle

(polar) head groups are oriented outward keeping themselves in contact with the bulk aqueous phase. Normal aqueous micelles are generally produced by single-chain surfactants as chain- branching inhibits micellization.

Micelles are considered to be dynamic in nature, with continuous exchange of surfactant molecules in and out of the aggregates occurring in the milliseconds to microseconds range. Thus, individual surfactant molecules (called monomers) are thought to be distributed throughout the aqueous phase surrounding the micelles or monomers and micelles are visualized as being uniformly distributed throughout the aqueous phase. When more than one surfactant are present in aqueous solution, mixed micellar system is produced. The surface activity of the mixed micellar system has been reported to be superior than the single surfactant micelle system (50).

b) ***Reverse Micelles*:** In contrast to the normal micelles, which are formed in polar (i.e. aqueous media) solvents, reverse micelles are formed in non-polar solvents like hexane or chloroform and a trace of water where the polar head groups of the surfactant are directed towards the interior of the aggregate and the hydrocarbon chains are in contact with the non-polar solvent. Compared to normal micelles, reverse micelles are more complex and less understood. Reverse micelles offer the same potential advantages for analysis as do normal micelles i.e. the ability to solubilize polar species that would be excluded from normal micelles. An interesting aspect of reverse micelles is their capability to solubilize water in the interior of micelle structure. In many instances water actually promotes the formation of larger and more stable micelles.

From macroscopic perspective, micellar solutions are homogeneous and cannot be filtered. However, the unique characteristics of micellar aggregates stem from their microscopically non-homogeneous nature i.e.

they provide a microenvironment which is distinctly different from the bulk solvent. The most important property of micelles is their ability to solubilize substances that are otherwise insoluble (or sparingly soluble) in water.

The use of micellar solutions as mobile phase in TLC was first suggested by *Armstrong et al.* (51,52) who described the possible advantages of micellar mobile phase (MMP) systems over traditional pure and mixed solvent systems. Using aqueous SDS solutions in combination with polyamide and alumina thin layers they successfully separated pesticides and the pollutant decachlorobiphenyl by normal-phase TLC. However, nucleosides could be separated by reversed–phase TLC using a reversed micelle solution as mobile phase. It was demonstrated that the partitioning of substances to the reversed micelles involved both electrostatic interactions with the polar head groups of the surfactants and the solubilization by the "*water pool*" in the hydrophobic core. Despite several advantageous features of MMP, its use in TLC has been less intense compared to HPLC (53). Micellar TLC has been used to separate substituted benzoic acids, polynuclear aromatic hydrocarbons and vitamins (54); phenols (55,56); amino acids (57-59); alkaloids (60); dyes (55, 60-62); aromatic amines (63); drugs (64) and inorganics (65,66).

The separation with MMP is based on selective dissolution of polar and nonpolar substances as a result of combined effect of electrostatic, hydrophobic and donor–acceptor interactions. However, inorganic MLC differs from organic MLC in the sense that in micellar liquid chromatography of inorganic ions, the electrostatic interaction is the primary or in most cases the only separation mode. The retention behaviour can be described with an ion exchange model, the retention is principally determined by the concentration and a counter-ion. On the other hand in micellar liquid chromatography of organic compounds, hydrophobic or lipophilic interactions, decide the retention pattern of solutes.

From the above discussion it looks that micellar mobile phases have much to offer to physical, biochemical and analytical chemists. Even a cursory look at the recent literature points out that MMP have been the focus of numerous studies related to chemical separations and all signals indicate this heightened interest will provide a powerful alternative to conventional hydro-organic eluents to solve difficult analytical problems. It is also perceived that TLC has more to offer to the study of micelles than MMP have to offer to chemical analysis performed by TLC. This aspect of micellar TLC will be a rewarding field for future research.

1.14 SAMPLE APPLICATION

Sample application is one of the most important steps in the technology of TLC. Improperly applied samples result in poor chromatograms. Samples are applied as spots or bands on the sorbent layer, about 2-3 cm above from the lower edge of the TLC plate so that only sorbent layer makes contact with the mobile phase and the sample does not dissolve in the mobile phase. The sample should be completely dried before placing the plate in the development chamber. Dilute solutions can be applied to the layer either with solvent drying between successive applications or after bringing the sample solution to proper concentration.

Micropipette, micro syringe, melting point capillaries etc. have been used to apply the sample on the plates. A number of automatic spotters of varying design are also available in the market.

1.15 DEVELOPMENT

Development in TLC is the process in which the mobile phase moves across the sorbent layer to effect separation of the sample substances. Ascending development or linear development is the commonly used mode of development in TLC in which the mobile phase moves up (ascends) the plate. Any close container that will hold the plate upright is usable. While performing the development one should take care of the angle of the development and saturation of chamber apart from other factors.

It has been observed that the angle of development, that is the angle at which the plate is supported, effects the rate of development as well as the shapes of the spots (67). An angle of 75^0 is optimum for development. If a desired separation is not achieved by simple development, (ii) stepwise development, (iii) continuous development, (iv) two dimensional development, (v) circular development and (vi) reversed–phase partition development, are available to achieve the desired separation.

1.16 VISUALIZATION

The methods of visualization (detection) used in TLC are of three major types, (I) physical (ii) chemical and (iii) enzymatic or biological. Among the physical methods, visualization in UV-light is most common. This method is highly sensitive, non-destructive, and amenable to the visualization of spots before undertaking quantitative studies. Chemical methods of detection involve the spraying of chromatoplates with a suitable reagent, which forms coloured compounds with the separated species. Reagents giving clear and sufficiently sensitive colour reactions with several species are preferred. Both selective and non-selective reagents may be chosen for the location of the separated zones. *Nanda* and *Devi* have reported an enzymatic method (68) for the detection of heavy metals in fresh water. *Nicolous* and *Coroneli* (69) have reported a microbiological method called bioautography for the detection of antibiotics on TLC plates using triphenyltetrazolium chloride (TTC) and a micro-organism that is sensitive to the antibiotic in organism that is sensitive to the antibiotic in question.

1.17 QUANTITATION

The three main approaches associated with quantitation in TLC are (i) visual estimation (ii), zone–elution and (iii) *in situ* densitometry. Amongst these, *in situ* densitometry is the preferred technique for quantitative TLC. Substances separated by TLC or HPTLC are quantified by *in–situ* measurement of absorbed visible or UV–light, measured either on regular layers or on layer with incorporated phosphor.

1.18 LATEST DEVELOPMENT IN TLC

As a result of the recent innovations, several new techniques such as high–performance thin layer chromatography (HPTLC), overpressurized thin layer chromatography (OPTLC), centrifugal layer chromatography (CLC), reversed–phase thin layer chromatography (RPTLC), radial chromatography, hot–plate chromatography, bioautoradiography, immuno–staning and enzyme inhibition techniques came into light. HPTLC layers being thinner and made of sorbent with more uniform particle size are developed for a shorter distance. All these factors led to faster separations, reduced zone diffusion, lower detection limits, less solvent consumption and better separation efficiency.

1.19 COMBINATION OF TLC WITH OTHER ANALYTICAL TECHNIQUES

The careful combination of TLC with other analytical techniques is more useful to collect information regarding the analysis of a complex sample. Spectrophotometer, high performance liquid chromatography and gas chromatography, in conjugation with TLC are the three most widely used techniques. However, mass/GC, infra red and thermal analytical techniques in combination with TLC have also been used. One of the newest techniques used in combination with TLC is photoaccoustic spectrometry, which is capable to locate compounds *in-situ* on the plate. *Issaq* and *Barr* (70) combined TLC with flameless atomic absorption spectrometry (FAAS) to identify an inorganic compound in an impure organometallic complex and to determine the recovery and purity of organometallic samples.

The examples cited above reveal, how the separation method of TLC complement the analytical methods necessary for the absolute identification of a substance. TLC provides an excellent purification method for separating substance of interest from other contaminants in the sample. Analytical techniques can then be applied to identify the separated substances.

Table 1.4

Literature on Thin-Layer Chromatographic Studies Performed During Last Twenty Years on Pesticides and Inorganics Using Soil as Stationary Phase.

Analyte	Remarks	Ref. No.
Pesticides	Development of a micro-extraction technique in combination with GC to establish relative mobility of some co-applied pesticides such as alachor, atrazine, carbofuran, cyanazine, ethoprop, metolachlor and metribuzin.	71
Organo phosphorus insecticide	Examination of migration trend of chlorfenvinphos, trithion, delnav and dichlofenthion through soil layer using insecticide (extracted in heptane) at concentration level of 150 or 1000 μg per plate. Quantitative determination by GC and detection by autoradiography.	72
Trace elements	Determination of R_F values of Fe^{2+}, Fe^{3+}, Cu^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+} in the presence and absence of soil organic matter, using aqueous solutions of electrolyte (conc. 0.1-1.0 N) as mobile phase. None of the metal ions showed any mobility with Na_2CO_3 eluent. Mobility of all metal cations was higher on layers prepared from organic matter free soil.	73
Trace elements	Investigation about the variation in the movement of Cu^{2+}, Fe^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+} as a function of salinity, alkalinity, phosphate content and organic matter of soil.	74
Agrochemicals	Studies on the effect of nature of soil on the mobility (or R_F values) of labeled agrochemicals (chlordimeform, Padan, MBC, CP, ASV etc.)	75

Analyte	Remarks	Ref. No.
Radio labeled pesticides	Studies of degradation of aryldiolkylthiourea herbicides into corresponding ureas on soil surface using soil-silica layer as stationary phase.	76
Pesticides	Study of the influence of organic matter, $CaCO_3$, fly ash, saline and alkaline salts, inorganic fertilizers, surfactants, and exchangeable ions on the mobility of five carbamoyl group containing pesticides (aldicarb, bavistin, carbofuran, dimecron and oxamyl).	77
Trace elements	Enhancement of the mobility of trace elements by removal of organic matter from soil and with the increase in the concentration of oil cake extracts.	78
Heavy metals	The mobility of some heavy metals was investigated using amino acids, organic bases, organic acids and their sodium salts as mobile phase, results were explained on the basis of reaction mechanism of complex formation, stability, molecular size, pH and the nature of the heavy metals.	79
Phosphorus containing pesticides	Examination of effect of pH, salt leachate concentration, inorganic fertilizers and surfactants and on mobility of DDVP, diazinon, ekatin, folithion, malathion, metasystox, parathion methyl and rogor by soil TLC using alkaline and saline salts, inorganic fertilizers and surfactants as mobile phases.	80
C^{14}-labeled pesticides	Examination of the mobility of ^{14}C-labeled pesticides using distilled water as mobile phase. The mobility of pesticides followed the order: aldicarb> metamitron> atrazine $\approx$ chloridazon> isobaxen.	81
Avermectin	Determination of mobility of Avermectin Bla (AVM) in soil (aged and unaged) by sorption/desorption using batch equilibrium technique, soil TLC and soil column leaching method. Avermectin Bla was found to be immobile (R_F=0.0) on both type of soil.	82

Analyte	Remarks	Ref. No.
Fluometuron	Examination of movement of fluometuron in soils with the aid of radioautograms of soil coated on TLC plates. Fluometuron has been found to be more mobile in soil containing low organic matter.	83
Cadmium	Examination of the mobility of cadmium in various natural soils. The results of soil TLC show the strong control of soil properties on the mobility of cadmium wastes from industrial mining and farming applications.	84
C^{14}-Labeled pesticides	Determination of pesticides mobilities by autoradiographs and linear analyzer. The pesticide mobility decreases in the order, acephate> fluometuron> atrazine> ethofumesate> diazinon> glyphosate.	85
Heavy metals	Examination of mobility of some heavy metals Ni, Mn, Cr, Cu and Pb using soil with decomposed organic matter as well as soils amended with commonly used pesticides (thiometon, malathion, dichlorovos, trichloroacetic acid, p-chlorophenoxyacetic acid and β-naphthoxyacetic acid etc.). The mobility of the heavy metals followed the order Ni> Mn> Cr> Cu> Pb in all the systems studied.	86
Labeled pesticides	Use of eleven different micro structural soils to study the mobility of pesticides. A new correlation i.e. $M=W_R R_F$ where M is pesticide movement, W_R is the rate of water movement and R_F is the retardation factor was proposed to understand the pesticide movement through soil layer under the action of rain.	87
Fungicides	Assessment of the movement of pesticides by soil TLC using successive elutions. Visualization of radiolabeled compounds using linear detector and autoradiography on glass plates layered with a fine soil fraction (sieved at 100μm) having layer thickness 250μm.	88

Analyte	Remarks	Ref. No.
Pesticides	Examination of leaching pattern of pesticides by soil packed column chromatography/soil TLC.	89
Pesticides	Investigation of migration behaviour of active pesticide ingredients on soil layer using water as mobile phase and their identification applying physico-chemical detection methods.	90
Radioactive pesticides	Determination of radioactive pesticides using linear analyzer to obtain both linear radiochromatograms and precise images in two or three dimensions of the spots on the plate.	91
Pesticides	Use of reversed-phase TLC in conjunction with video densitometry for the determination of a six-component mixture of pesticides. Methanol-water solvent systems were used for achieving separation of propham, chlorpropham, atrazine, diflubenzuron, tetramethrin and α-cypermethrin.	92
Trace metals	Investigation of effect of some organic compounds on the mobility of trace metals viz; Co., Zn, Cu, Ag and Pb through soil amended with flyash. The results indicate decrease in the mobility of trace metals with increasing dosage of flyash in soil but with a variation in intensity by the use of organic compound as mobile phase (e.g. acetaldehyde, acetone, formaldehyde, ethyl methyl ketone etc.).	93
Pesticides	Study of chromatographic behaviour of some pesticides on silica, soil and mixed layers containing soil, with aqueous ammonium or sodium salt solutions, with or without added N-cetyl-N,N,N-trimethylammonium bromide (CTAB). Interesting aspect of this study is migration of pesticides such as phosphamidon and dimethoate through pure soil.	94

Analyte	Remarks	Ref. No.
Metal cations	Identification of best TLC system for metal cations separation from their multicomponent mixtures on soil mixed with silica layers. N-Cetyl-N,N,N-trimethylammonium bromide (CTAB) with edit urea was found to be the most favourable mobile phase.	95

Table 1.5

*Literature Arranged in Chronological Order on Thin-Layer Chromatographic Studies Performed During the Years 1991-2002 on Amines, Phenols, Inorganic Ions and Metal Complexes Using Layer Materials Other Than Soil As Stationary Phase.

Analyte	Remarks	Ref. No.
Several anines and their derivatives	Indole has been successfully separated from carbazole and diphenylamine using silica gel, alumina, cellulose and copper sulphate impregnated silica gel as stationary phase and several aqueous and non-aqueous solvent systems as mobile phase.	96
Alkyl and aryl amines	Use of methanol-water (1+1) with added salts and various buffers as mobile phases for the determination of lipophilicity of amines with reversed-phase TLC.	97
Substituted phenolic compounds	Good separations of substituted phenolic compounds on silica gel layer containing F_eCl_3 or $K_3F_e(CN)_6$.	98
Tris (β-diketonato) complexes of Co^{3+}, Cr^{3+} and Ru^{3+}	Use of single and multicomponent organic and aqueous-organic solvent systems for qualitative analysis of metal complexes on silica gel or silica gel $^{60}F_{254}$ HPTLC plates.	99
Forty-nine inorganic ions	Use of aqueous H_2SO_4 (0.01-1.0M) and H_2SO_4 + ammonium sulphate systems as mobile phase and p-aminobenzyl cellulose as stationary phase for qualitative separations.	100

**Inorganics, amines and phenols have been clubbed together in order to maintain the chronological order of literature.*

Analyte	Remarks	Ref. No.
Inorganic ions	Selective separation of Re(VII) from many inorganic ions using aqueous HCl and HCl+ ammonium chloride systems as mobile phase and diethyl-(2-hydroxypropyl)- aminoethyl QE-cellulose as stationary phase.	101
Zrand Hf	Silica gel as stationary phase and HNO_3 + HCl or H_2SO_4 containing different concentrations of hydrogen peroxide as mobile phase were used to achieve complete separation of Zr from mixtures containing Zr+Hfratios ranging from 20+1 to 1+40.	102
Several metal ions	Eleven neutral and acidic solvent systems were used as developer in combination of cellulose and synthesized carbamide formaldehyde polymer (aminoplast) layers for achieving separation of metal ions of different valency states.	103
Fe,Ni,Zn,Cu, Pb and Mn	Solvent systems comprising of aqueous solutions of sodium thioglycolate (0.01-0.2M) were used in combination with silica gel 'G' plates for the determination of separated metal ions by atomic absorption spectroscopy.	104
Forty-nine inorganic ions	Aqueous HCl and HCl-ammonium chloride mixtures were used as mobile phases with p-aminobenzyl cellulose layers for qualitative TLC.	105
Ce^{2+}, Ce^{4+}, Nd, Eu, Gd, Tb, Yb, Y, Ti, V, Zr and Th.	An increase in the R_F values of the lanthanide ions was observed on increasing the concentration of citric acid in the mobile phase, on layers of silica gel coated with different concentrations of primine JM-T.	106
Alkali metals	Qualitative separations were achieved on zinc ferrocyanide layers using aqueous ammomium nitrate as mobile phase.	107

Analyte	Remarks	Ref. No.
Rare-earth elements	Use of solutions of acids, bases and salts as mobile phase and diatomite as stationary phase for getting qualitative separations.	108
Cu, Co, Cd, Hg, Ni and Ag.	Qualitative separations and determination of chromatographic parameters as a function of the concentrations of MeOH, NH_3, and inorganic salts in the mobile phase. chitin/chitosan was used as stationary phase.	109
Ni, Cu, Zn, Pd, Cd, Cr, Fe, Ru, Rh, La, Au, Tl, Zr, Pt, Nb, Ta, Mn, Ag, Hg, Co, Mo and W	A correlation between R_F values and atomic numbers of the metal ions was noticed on silica gel layers impregnated with DMSO and developed with DMSO+THF (1+10).	110
Transition metal ions	Qualitative TLC was performed on chitin/chitosan layers using several aqueous mobile phases.	111
Twenty-one inorganic cations	Separation and identification of cations on cellulose layers using acetylacetone+acetone+conc. HCl (5+5+1) mobile phase.	112
IO_3^-,IO_4^-, BrO_3^-, I^-, MoO_4^{2-} and $Fe(CN)_6^{4-}$	Examination of effect of heavy metals on the chromatographic separation of periodate from other oxyanions and cyanoferrates on silica gel layers developed with distilled water.	113
Eighteen anions	Investigations of the effect of transition metals on Cl^-– Br^-– I^- and NO_2^-–NO_3^- separations on alumina or alumina added silica gel layers developed with mixed acidic-organic solvents containing formic acid.	114

Analyte	Remarks	Ref. No.
Seven anions	Use of anhydrous antimony (V) oxide as stationary phase and aqueous organic acids as mobile phase for achieving qualitative separations.	115
Halides, oxyanions, hexacynoferrate, thiocyanate and phosphate	Microgram detection and separation of anions on silica gel impregnated with aqueous salt solutions of Cu, Zn, Ni or Co and developed with acetone mixed with DMSO, formic or mineral acid.	116
Several amines	Separation and determination of nature of colour of spots, lowest detectable amount and R_F values of amines on 1% sodium carboxymethyl cellulose added silica gel layers developed with mixed organic solvents containing acetone.	117
Ten aromatic and aliphatic amines	Use of seven aqueous systems to illustrate that an increase in the hydrophobic part of amines results in their increased retention on polyacrylonitrile layer.	118
Twenty aromatic amines	Effect of impregnant concentration, type of mobile phase and concentration of organic solvent in the mobile phase on the mobility of amines was investigated using silica gel impregnated with ammonium cerium (IV) nitrate layers as stationary phase and various organic solvents at different concentration levels as mobile phase.	119
Several aromatic amines	Use of 5-chloro-4,6-dinitrobenzo-furazon as a new chromogenic reagent for highly sensitive detection of aromatic amines on TLC plates.	120
Phenolic compounds	Determination of phenolic compounds in pharmaceuticals using MeOH+xylene (1+1) as developer.	121

Analyte	Remarks	Ref. No.
Phenols and naphthols	Use of silica 60 plus kieselguhr F_{254} and polyamide as layer materials for detection with preliminary separation of phenols using seven organic chromogenic reagents.	122
Fe, Cu and Mn	Qualitative separations were achieved on cellulose layers developed with ethanol+isobutanol+conc. HCl+water solvent systems.	123
Pb, Cd and Zn	Determination of heavy metals by TLC-square-wave anodic stripping voltammetry using microcrystalline cellulose.	124
Mn, Co, Ni, Cu, Zn, Fe, Cr, Ti and V	Qualitative identification of 3d metal ions by reversed-phase TLC on layers prepared from silica gel coated with high molecular weight amines and developed with aqueous succinic acid.	125
Eleven metal ions	Study of retention behaviour of metal ions from aqueous solutions (pH1-7) on alizarin red S+aliquat 336 impregnated silica layers.	126
Metal ions	Sixteen different solvent systems were used to develop plates prepared from mixtures of silica and inorganic ion-exchanger gels for achieving qualitative separations.	127
Mg, Al, Ca, V, Cu, Zn, Ge, Y, Zr, Mo, Ag, Cd, In, La, Ce, Eu, Tb, Tl, Pb and Bi	Detection at picogram level of fluorescent cations after separation on porous glass sheets using n-butanol+benzene+1M HNO_3+1M HCl (75+9+4+2, $^{v}/_{v}$) or acetone + 3.0M HCl (99+1, $^{v}/_{v}$) as mobile phase.	128
Forty-nine inorganic ions	Separation of Se(III), rare-earth(III), Y (III), Th(IV) and V(VI) from other ions, on diethyl-(2-hydroxy propyl) amino ethyl cellulose layers developed with aqueous sulphuric acid and sulphuric acid-ammonim sulphate.	129

Analyte	Remarks	Ref. No.
Several aromatic amines	New analytical technique (TLC-spectrophotometry under the influence of temperature gradient) for the simultaneous determination of aromatic amines after separation on silica gel plates.	130
Fourteen amines	The comparative study of mapping derivatised structurally diverse amines by two-dimensional TLC.	131
Fourteen chiral amino alcohols and amines	Use of RP-18 WF_{254} HPTLC plates for separation of chiral amino alcohols and amines after derivatization with Marfey's reagent.	132
Phenols and quinolines	Use of silica gel TLC plates for the examination of retention pattern of phenols and quinolines developed with dioxane or ethylacetate mixed with heptane.	133
Fe, Co, Zn, Cd, Cu and Ni.	Use of silica gel modified with analog of dibenzo-18-crown-6 as stationary phase for the analysis of alloys and natural water samples.	134
Rare-earth elements	Preconcentration of rare-earths by circular TLC for subsequent ICP-AES determination in geological samples on fixion 50x8 layers developed with 0.1M $H_2C_2O_4$, 2.0M NH_4Cl, 5.0M HCl or 0.5M ammonium citrate.	135
Toxic metals	Normal-phase, reversed-phase and chelation TLC of metal ions. Quantitative separation of Pb from synthetic alloys using stationary phase prepared from plain silica gel loaded with various concentrations of EDTA or TBP, developed with DMSO-HNO_3 and DMSO-HCl systems.	136
Twenty-six transition and alkali metal ions	Qualitative separations on cellulose layers.	137
Heavy metals	Stannic sulfosalicylate layers were used for achieving quantitative separations of Fe(III), Cu(II) and Pb(II) from other metals.	138

Analyte	Remarks	Ref. No.
Some anions	Separation of anions in the presence of hardness causing salts. Identification of NO_2^- in artificial sea water, using silica gel 'G', alumina, cellulose microrystalline and alumina+silica gel 'G' stationary phases.	139
Cl^-, Br^-, I^-, ClO_3^-, ClO_4^-, $H_2PO_4^-$, NO_2^-, NO_3^-, SCN^- and SO_4^{2-}	Use of acetone+chloroform mobile phase in the ratio 3:1 for achieving separation of inorganic anions as diantipyrilmethane using radial or ascending technique.	140
Diphenylamine and its nitrate derivatives	Application of TLC for isolation and identification of diphenylamine and its nitrate derivatives from smokeless gun powder samples.	141
Thirteen aromatic amines	TLC of aromatic amines using surfactants in stationary as well as in the mobile phase. Best separations were with anionic surfactant (SDS).	142
Phenols	Separation of phenols by normal and reversed-phase TLC using RP–8 and RP–18 silica gel layers developed with mixed organic solvents containing benzene.	143
Phenols	Examination of mobility of phenols by normal and reversed–phase TLC using RP–8 and RP–18 silica layers developed with mixed organic and aqueous–organic solvents.	144
Forty–nine inorganic ions	Use of aqueous sulphuric acid and sulphuric acid+ammonium sulphate mobile phase with arsenosilicates of Sn(IV), Cr(III) and Sb(V) layers for demonstrating an increase in R_F values with increasing acid or sulphate concentration in the mobile phase.	145

Analyte	Remarks	Ref. No.
3d Series transition metal ions	Examination of mobility pattern of metal ions as a function of concentration of HNO_3 on silufol layers.	146
Uranium	Selective separation of uranium from synthetic mixture of several metal ions on TLC plates prepared from silica gel impregnated with high molecular weight amines and developed with mixtures of DMF and HNO_3 or HCl.	147
Cations with some anions	Qualitative separation of cations and anions on silica gel impregnated with mono–2–ethyl hexyl acid phosphate layers developed with aqueous MeOH containing tributylphosphate and formic acid.	148
Inorganic ions	Use of silica gel impregnated with mono–2–ethyl hexyl acid phosphate as stationary phase and sulphuric acid and organic solvents as mobile phase for achieving separation of polyvalent ions and trivalent rare–earths which tend to form anionic sulphate complexes.	149
Inorganic metal cations	Study on migration behaviour of metal cations under the influence of pH of mobile phase and the concentration of impregnants.	150
Copper	TLC of Cu after extraction from biological tissues by dry oxidation on silica gel 'G' plates developed with MeOH+ acetic acid.	151
Hg, Cu and Cd	Use of benzene+acetone+DMF (5+4+1) mobile phase with silica gel 60 layer for UV spectroscopic determination of metal ions after elution with H_2O.	152

Analyte	Remarks	Ref. No.
Phenolic compounds	Separation of different phenolic compounds from rapseeds on silica gel, cellulose, octadecyl silica layers developed with benzene+methanol+acetic acid (45+8+4).	159
Inorganic ions	Use of isobutyl methyl ketone (IBMK) + formic acid (FA) mobile phase and silica gel TLC plates for examination of relationship between concentration of IBMK/FA and the R_F values of ions.	160
Inorganic ions	Use of microcystalline cellulose as stationary phase and HCOONa (1.0M)+KI (1.0M) in 1:9 ratio as mobile phase for the study of retention behaviour of inorganic ions.	161
Rare–earths	Decrease in the R_F values of metal ions was noticed with increasing pKa value of amine used for pretreatment of silica gel layers developed with NH_4Cl solution.	162
Actinides	Separation on the basis of different sorption behaviour of actinides in their ter–and penta–valency states on silica gel impregnated with polyethylene glycol.	163
Heavy metals	Qualitative separation and detection of heavy metal cations on silica gel and alumina layers impregnated with LiCl and developed with formate ion containing eluents.	164
Cd, Zn, Cu and Pb	Quantitative separation of Cu(II) by AAS after TLC separation from other metals on silica gel layers using HCOONa (1.0M)+KI(1.0M) in 1+9 ratio as mobile phase.	165

Analyte	Remarks	Ref. No.
Au, Ru, Rd, Pd, Dy and Pt	Evaluation of resolution for several pairs of ions and the estimation of their detection limits on silica gel plates developed with HCl+acetylacetone solvent systems.	153
Twenty–seven aromatic amines	Qualitative analysis and examination of effects of the nature of substituent groups and the number of C–atoms in the molecule of amines on the retardation factor of various amines on silica gel 'G' or silica gel impregnated with aqueous solutions of various sodium salts developed with cyclohexane+benzene (1+4) solvent system.	154
Nitrosoamines and amines	Application of computer–aided optimization technique for sample clean up of nitrosoamines and amines by solid phase extraction on diol–and CN– bonded sorbent plates developed with cyclohexane–dioxane, heptane, EtOAc, iso–octane, cyclohexane or 2–propanol.	155
Eight biogenic amines	Determinations and separation of biogenic amines in fish samples by two dimensional TLC using benzene+triethylamine (5+1) and benzene+ triethylamine+ acetone (10+2+1) mobile phases.	156
Several aromatic amines	Identification and separation of aromatic amines on silica gel, alumina, cellulose, cellulose+silica gel (4+1) layers developed with 1% aqueous and salt solutions.	157
Phenols	Use of impregnated and non–impregnated silica gel layers developed with benzene+diethylamine (4+1 or 7+3) solvent systems for achieving qualitative separations.	158

Analyte	Remarks	Ref. No.
Transition metals	Separation of eight–components mixtures of transition metal cations on silica gel impregnated with EDTA, dimethylglyoxime or 1,10–phenanthroline layers developed with pyridine + benzene+acetic acid + H_2O (6+5+8+4, 5+5+4+1) and BuOH+ benzene + formic acid (5+10+9) and quantitative estimation by AAS.	166
I^-, IO_3^-, IO_4^-, Br^-, BrO_3^-, NO_2^-, SCN^-, CrO_4^-, PO_4^{3-}, MnO_4^- and WO_4^{2-}	Silica gel impregnated with $CuSO_4$, alumina, cellulose containing alumina or kieselguhr stationary phase and distilled water, aqueous HCOOH or HCOONa and acetone plus HCl mobile phase were used for achieving semiquantitative determination of I^-, Br^-, and NO_2^- by spot-area measurement.	167
I^-,IO_3^-, IO_4^-, Br^-, BrO_3^-, NO_2^-, MnO_4^- and CrO_4^{2-}.	Silica gel, cellulose, alumina 'G', kieselguhr and their mixtures were used as adsorbents for semiquantitative determination of IO_4^-, by peak height measurement using water-in-oil microemulsion as developer.	168
Twenty–six primary aromatic amines	TLC of primary aromatic amines and their qualitative separations on iron (III) tungstophosphate layers developed with aqueous acidic and salt solutions.	169
Several amines	Detection of trace quantities (≈ng) of amines on TLC plates with the use of enzymatic reactions.	170
Natural phenolic derivatives	Studies of chromatographic properties of phenolic derivatives on silica gel plates using mobile phases of different polarities.	171
Phenols	Separation and quantitative determination of pentachlorophenol in leather goods on aluminium sheets (RP–18 F_{254}).	172

Analyte	Remarks	Ref. No.
Thirty cations	Selective separation of Pt on Ce(III) silicate layers developed with fifteen solvent systems including NH_4OH (0.5M).	173
Cd, Cu and Pb	Evaluation of detection limits of Cd and Pb with preliminary separation by TLC.	174
Heavy metals	Identification of metals in human bones, placenta, milk and air by adsorption and IE–TLC.	175
Ni, Co and Cu	Determination of metals in rock samples by TLC/photodensitometry.	176
Co, Fe and Cu.	TLC of metal ions by the use of plasma polymerization technique for coating impregnant (sodium salt of condroitin sulphate) on silica gel layers which were developed with aqueous or organic solvents of different pH values.	177
Al, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sn, Ta, Ti, V, Y and Zr.	ICP–AES determination of Zr in Zr–U alloys after separation by TLC on TBP coated polymeric supports.	178
Metal cations	Determination of Fe in process media by employing 8–hydroxy quinoline as complexing agent.	179
Metal cations	Use of 8–hydroxy quinoline as complexing reagent and lumogallion as detector for Al.	180
Mo, V and W	Selective separation of Mo from vanadium and tungsten on alumina layer.	181

Analyte	Remarks	Ref. No.
Fe and Cr	The separation of Fe^{3+} and Cr^{3+} as well as some anions of elements on molecular sieves NaX layers.	182
Nine–anions and eleven cations	TLC separation and colorimetric determination of SCN^- in water and wastewater on cellulose microcrystalline, kieselguhr and cellulose plus kieselguhr layers developed with NH_4OH (1.0M) + acetone (1+9, 3+7, 1+1, 7+3, 9+1).	183
Twenty–eight metal ions	Use of lanthanum silicate ion exchanger as adsorbent for achieving separation of several metal ions from their multicomponent mixtures.	184
Sixty–four ions	Selective separation of Zr(IV), Hf (IV) and many other ions on silica gel plates developed with HNO_3 and HNO_3+H_2O_2.	185
Metal ions	Quantitative separation of Mo^{6+} after separation from Cr^{3+}, Cu^{2+} Fe^{3+} and Co^{2+} on stannic selenite silicate layers developed with DMSO–HNO_3 systems.	186
Toxic metals	Selective qualitative separations of toxic heavy metals.	187
Several bi–,tri–, tetra– and penta–valency ions	Selective separation and identification of metals of different valency states on microcrystalline cellulose and silica gel layers.	188
Metal complexes	The application of TLC in the detection and separation of Ni and Co complexes.	189
Metals and minerals	Detection of trace amounts of metals and minerals.	190

Analyte	Remarks	Ref. No.
Sixteen primary aromatic amines	Qualitative and quantitative analysis of primary aromatic amines on zirconium molybdophosphate mixed with silica gel 'G' layers developed with sodium nitrate and hydrochloric acid solutions.	191
Several amines	Determination of chlorphenamine meleate in Xiaojieling granules by scanning the spot at 264 nm appeared on silica gel F_{254} TLC plates, developed with ethylacetate+methanol+ 6% acetic acid (5+3+2). The recovery and RSD were 98.2% and 3.5% respectively.	192
Phenolic acids	Selective separations were achieved using ethylacetate + formic acid + acetic acid + water (100+11+11+27, v/v) and ethyl acetate+FA+H_2O, (8+1+1, $^{v}/_{v}$) mobile phases.	193
Carboxylic acids, phenols and related compounds	Selective separations of phenols and related compounds were achieved using chloroform+ethylacetate+acetic acid (50+50+1) mobile phases.	194
Phenols	Phenols were detected and separated on silica gel, silica gel plus kieselguhr and polyamide layers.	195
Phenols	Use of stationary phases with bonded amino–, cyano–and diol-groups in the analysis of phenols with chloroform+2–propanol (50+1.5 and 50+4, $^{v}/_{v}$), solvent systems.	196
Phenolic acids	Selective separation of phenolic acids on diol-bonded silica gel layers developed with n–heptane, ethylacetate, isopropanol, dioxane or THF.	197

Analyte	Remarks	Ref. No.
Pb and Cd	Separation of lead and cadmium from humic acid.	198
Metal chlorosulphates	Examination of mobility and selective separation of metal chlorosulphates or chicken egg shell powder alone and mixed with cellulose or silica gel 'H' layers developed with acid containing mobile phases and aqueous ammonium sulphate solution (1.0M).	199
Thirty-three metal ions	The study of retention sequence of metal ions on silica layers developed with methyl isobutyl ketone and formic acid.	200
d–Block metals	Binary and ternary separations were achieved on stannic phosphate silicate layers developed with buffered EDTA solutions.	201
Metal cations	Separation and identification of ten cations on microcrystalline cellulose layers.	202
Cr and Ni.	Determination of Cr and Ni in high steel after TLC separation on microcrystalline cellulose layers developed with acetone+hydrochloric acid+water (43+4+3, $^{v}/_{v}$).	203
Heavy metals	Separation and quantification of individual metal from a five-component mixture of heavy metal ions on silica gel impregnated with EDTA (2%) using tetra ammonium bromide (1%) plus phosphate buffer (pH 8.0, 2.0M) as mobile phase.	204
U and Th.	Separation and determinationm of U and Th (conc. range 2.5–3.0μg) in the presence of other metal ions using isopropyl dithiophosphoric acid as mobile phase.	205

Analyte	Remarks	Ref. No.
Twenty inorganic cations	Detection of inorganic cations by two–dimensional TLC on microcrystalline cellulose layers developed with 1–butanol saturated with a 1+1 mixture of 3.0M HNO_3 and 1.0M HCl and MeOH+36% HCl (10+3, $^{v}/_{v}$).	206
Toxic metals	Separation of Cd^{2+}, Pb^{2+}, Bi^{3+}, Hg^{2+}, Co^{2+} and Cu^{2+} on cellulose MN, silica gel modified with nercaptopropyl trimethoxysilane and silica gel 'R' impregnated with piperazine, developed with lower alcohols and benzene.	207
1,3–Diketonates of heavy metals	The separation of 1–3 diketonates and other metal ions on silufol and plasmachrom plates using micellar solutions containing sodium dodecyl sulphate as eluent.	208
Biogenic amines	Use of silica gel TLC plates for separation and in-situ densitometric determination of biogenic amines as dansyl derivatives in food samples.	209
Isomers of aromatic amines	Qualitative separation of m–isomers of aromatic amines from corresponding o– and p–isomers that moved faster giving higher R_F values, on alumina, cellulose, silica gel 'G' and silica gel 'H' layers developed with water–in–oil microemulsion consisting of SDS or CTAB, water, heptane and 1–pentanol or butanol.	210
Several aromatic amines	Adsorption (with isopropanol–n–hexane eluent) and partition (with methanol–water eluent) TLC separation of aromatic amines which are the reduction products in silica gel and reversed– phase layers.	211

Analyte	Remarks	Ref. No.
Biogenic amines	Quantitative analysis of biogenic amines present in fish meals using silica gel $^{60}F_{254}$ layers as stationary phase and dichloromethane+trimethylamine (10+1) as developer.	212
Primary and secondary aliphatic, fatty aromatic and heterocyclic amines	Use of isonitrosoacetyl acetone mobile phase for qualitative analysis of amines derived from acetone.	213
Biogenic amines, alkaloids and their derivatives	Adsorption and partition chromatography on non-bonded silica gel, bonded amine or diol-silica plates and RP-18 layers developed with binary mobile phases composed of acetate buffer and organic solvents.	214
Phenolic acids	Use of silica, alumina and polyamide layers and non–aqueous mobile phase (n–heptane, 2–propanol, dioxane, THF or ethylacetate) for detection of phenolic acids with preliminary separation.	215
Phenolic acids	Qualitative separation and quantitative determination of phenolic acids.	216
Anilines, quinolines and phenol derivatives	Examination of retention behaviour of aromatic compounds on silica gel layer modified with inorganic salts and developed with dioxane, ethylacetate, benzene, dichloromethane and chloroform.	217
Heavy metal cations	Selective separation of heavy metal cations by micellar TLC on microcrystalline cellulose layer.	218
Hg, UO_2^{2+}, Fe, Pb, Cd and Zn	Identification of Hg^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+} in synthetic sludge by micellar TLC using silica gel impregnated with KSCN as stationary phase and aqueous formic acid–sodium chloride solvent systems as eluent.	219

Analyte	Remarks	Ref. No.
Co and Cr complexes	Some qualitative separations were achieved on polyacrylonitrile layer.	220
Co and Ni.	Mutual separation of Co^{2+} and Ni^{2+} on silica gel 'G' plus stannic arsenate gel (10:1, $^{w}/_{w}$) layer impregnated with 0.2M tributyl phosphate and developed with 1.0M aqueous potassium thiocyanate.	221
Thirteen metal ions	Detection and separation of heavy metal cations on reversed-phase tributylamine impregnated mixed layer composed of silica gel and Sn(IV) arsenosilicate using methanol containing mobile phases.	222
Several metal ions including Ag.	Selective separation of Ag^{+} from binary, ternary and quaternary mixtures of metal ions on thin-layers of microcrystalline cellulose, alumina 'G' and their binary mixtures developed with ammonia, acetic and sodium or ammonium salt solutions.	223
Cu^{+}, Cu^{2+} and Co^{2+}	Use of silica gel impregnated with metal salts for study of mobility sequence of colourful salts specially Cu(I), Cu(II), Co(III) salts.	224
Forty–three inorganic ions	Qualitative separations of inorganic species on layered double hydroxides. A new parameter SR_F was introduced to quantify the separating power of the sorbent.	225
Co^{3+} diamine complexes	Salting-out TLC to understand the mobility sequence of Co(III) complexes on silica gel, cellulose and polyacrylonitrile layers developed with aqueous solutions of ammonium sulphate at various concentration levels.	226

Analyte	Remarks	Ref. No.
Amine compounds	Detection of separated amine compounds using 4–chloro–5, 7–dinitrobenzofurazan and 7–chloro–4, 6–dinitrobenzofurazan as spray reagents.	227
p–Aminophenol	Determination of p–aminophenol in various medicinal forms by spectrophotometry coupled with TLC.	228
Secondary and tertiary amines	Identification of twenty amines on silufol–254–UV layers. Dependence of R_F on [ammonia]/[C_6H_6] ratio and on amine's polarity.	229
Biogenic amines	Extraction and purification of histamine and tyramine from homogenized fish meat using MeOH mobile phase.	230
Aromatic amines	Separation of p–dimethylaminobezaldehyde from p–dimethylamino cinnamaldehyde after their derivatization with diphenylamine on silica gel 'G', alumina, kieselguhr and microcrystalline cellulose layers using carbon tetrachloride, cyclohexane and diethylether as mobile phases.	231
Phenols and its derivatives	The TLC analysis of phenols and their selective separations on chitin layers developed with mixed aqueous-organic (aliphatic alcohols or low organic acids) mobile phases.	232
Nineteen phenols	Use of silica gel 'G' or silica gel 'G' impregnated with 0.001 to 0.1M EDTA as stationary phase and 1% CTAB in 0.04M boric acid+0.04M phosphoric acid+0.24M NaOH (50+50+8, $^{v}/_{v}$) as mobile phase for TLC analysis of phenols.	233

Analyte	Remarks	Ref. No.
Metal ions	Separation of metal ions from their binary mixtures using TLC plates coated with mixed oxides.	234
UO_2^{2+}, Cu, Ni and Cd	Separation of Cd^{2+} and UO_2^{2+} from Cu^{2+}, Co^{2+} and Ni^{2+} from their binary, ternary and quaternary mixtures on silica gel TLC plates developed with mixtures of acetone, water and acetic acid or HCl.	235
d–and–f–Block metal ions	BuOH–8.0M HNO_3 and aqueous HNO_3 were used as mobile phase for quantitative separation of Zr^{4+} and W^{6+} from binary mixtures and from synthetic alloy component systems on plain as well as TBA impregnated silica gel 'G' layers.	236
Cu, Ni, Co^{2+}, Co^{3+}, Fe and their 1,3–diketonates	TLC separation of metal ions and their 1,3–diketonates on silufol and RP–plazmachrom layers developed with 2.5 x 10^{-2}M aqueous SDS.	237
Thirty metal ions	Rapid separation of Al^{3+},V^{5+}, Hg^{2+}, Cd^{2+} and other ions from numerous metal ions on titanium (IV) silicate ion–exchanger layers.	238
Co, Ni and Cu	Reversed-phase TLC for separation and identification of coexisting Co^{2+}, Ni^{2+} and Cu^{2+} on stannic arsenate ion-exchanger + silica gel (10+1, w/w) impregnated with 0.2M TBP layers developed with 0.1M KSCN.	239
Metal ions	Identification and separation of Zn^{2+}, Cd^{2+}, and Hg^{2+} on silica gel TLC plates developed with surfactant containing mobile phase systems.	240

Analyte	Remarks	Ref. No.
I^-, IO_3^- and IO_4^-	Separation and determination of iodide and its oxyanions on aluminium oxide 'G' layers developed with mixtures of NH_4OH and CH_3COCH_3 in 1+9, 3+7, 1+1, 7+3 and 9+1 ratios.	241
Fourteen heavy metal cations	Studies on sorption behaviour of metal ions by normal–phase and reversed-phase TLC using layers prepared from silica+zirconium tungstophosphate gels.	242
Aromatic amines	Examination of migration behaviour of aromatic amines on silica gel TLC plates using non–ionic surfactant containing mobile phase systems.	243
Aromatic amines	Separation of indole from p–dimethylaminobenzaldehyde and diphenylamine on silica layer using CTAB-alcohol-water systems as eluent.	244
Phenolic compounds	Presentation of the most important work dealing with the polyphenolic compounds taking into account of different aspects related with analytical chemistry of these compounds.	245
Toxic metals	Analytically important separations of toxic metals on mixed bed of oxides of Ti and Si.	246
Metal peptidoglycan monomer complexes	Quantitative TLC, identification and separation of some metals and their peptidoglycan monomer complexes on cellulose layers.	247
Mg and Al	Ion–exchange TLC separation of Mg and Al. Quantitative estimation of Mg in Al–alloys on layers prepared from amberlite IRP–69 and microcrystalline cellulose mixed in different ratio and developed with 0.5–2.0M HCl and $HN0_3$.	248
Inorganic ions	Quantitative separation of IO_3^- from NO_2^- and BrO_3^-; quantitative estimation of IO_3^- on mixed stannic arsenate+alumina layer (1+9) developed with tri–n–butylphosphate-water-formic acid system.	249

Analyte	Remarks	Ref. No.
Chlorosulphates of Mn, Fe, Co, Ni, Cu and Zn	Separation of nickel chlorosulphate from manganese, iron, copper or zinc chlorosulphate on silica gel+cellulose (2+1, $^{w}/_{w}$) layers developed with distilled water.	250
Au, Ag, Cu, Ni, Cd, Cr, Hg, and Zn	Thin–layer chromatography coupled with spectrophotometry and titrimetry for quantitative separation of Au^{3+} and Ag^{+} from accompanying metal ions on silica gel 'G' and alumina 'G' layers developed with aqueous CTAB (1.2M) and aqueous $(NH_4)_2\ SO_4$ (2.5M).	251
Aromatic amines	Use of cationic and non-ionic surfactant-mediated systems as mobile phase in thin-layer chromatographic separation of aromatic amines on silica gel layers. A mobile phase water and (CTAB+methanol, 1+2, w/v) in 99.5+0.5 ratio was identified as the best solvent system for rapid separation of indole from p-dimethylaminobenzaldehyde and diphenylamine in the presence of cations and phenols.	252
Coinage metal cations	Chromatography of sixteen metal cations has been performed on silica gel layers with micellar mobile phases containing sodium dodecyl sulphate (SDS), an anionic surfactant.	253
Co(II) and Co(III)	Application of TLC-MALDI-TOFMS for identification of Co(II) and Co(III) acetylacetonates. The separated spots on the TLC plates gave similar mass spectra to that of the conventional MALDI-TOFMS when a mixture of solvents with acetylacetone was used as the developing and eluting solvents.	254
Heavy metal cations	The analytical potential of iso octylphenoxypolyethoxy–ethanol (Triton X-100), a non-ionic surfactant, is used as a mobile phase in the thin-layer chromatographic separation of heavy metal cations on silica gel layers. TX-100 (0.02M) at pH2.3 was found to be best mobile phase for the separation of heavy metal cations. The proposed method was successfully applied for simultaneous detection of Zn^{2+} and Cd^{2+} from a spiked human blood sample.	255

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CHAPTER-II

Thin-layer chromatographic studies on mobility of some pesticides through different soil containing static flat-bed phases.

2.1 INTRODUCTION

Flat bed or planar chromatography has been considered as one of the simplest analytical techniques with general efficiency for the separation of organic and inorganic substances (1-6). The convenience and cost effectiveness of thin layer chromatography (TLC) have resulted in its wide range of applicability in separation and identification of pesticides and related agrochemicals (7-13). In fact TLC and HPTLC techniques complement the other more sensitive and precise primary methods (e.g. GLC, HPLC, SFC and capillary electrophoresis) used for the analysis of pesticides. Most of the reported TLC methods involve the use of mixed organic solvent systems containing benzene, hexane, chloroform, acetonitrile, methanol, ethyl acetate or dichloromethane as one of the components. Of the layer materials examined, silica gel and alumina have been the most favoured. A few studies have also been reported (7–9) on the use of barium or calcium sulphate, calcium carbonate or phosphate, soil, cellulose, C18 bonded silica gel and calcium sulphate mixed with silica gel as layer material to examine the mobility pattern of pesticides. Investigations (14-18) regarding pesticides metabolism in soil and plants, uptake of pesticides by plants from soil and pesticides migration in soil by TLC indicate that soil TLC has much to offer to the chemists interested in examining the uptake, translocation and degradation of pesticides in the environment.

The present study was taken up with the aim of understanding the mobility pattern of some pesticides through a static-flat phase of soil in contact with pure water, aqueous salt solutions, organic solvents and aqueous surfactant solutions. The aqueous systems selected as mobile phase in the present study are generally encountered with the soil surface and hence the results of transportation of pesticides through soil bed under the selected experimental conditions will be helpful to formulate the strategy for preventing the migration of harmful pesticides into soil bed. In addition, we have also examined the mobility of pesticides through

amended soil with silica gel, alumina and cellulose to find out the effect of additives on the mobility sequence of pesticides.

2.2 EXPERIMENTAL

Apparatus:

A thin-layer chromatography apparatus (Toshniwal, India), 20 x 3.5 cm glass plates and 24 x 6 glass jars were used.

Chemicals and Reagents:

Silica gel G (Merck, India) and N-cetyl-N,N,N-trimethyl ammonium bromide (CTAB) of CDH, India were used. All other reagents (cellulose, kieselguhr, ammonium sulphate, sodium chloride, methanol, toluene, cyclohexane, ethyl acetate, benzene etc.) used were also of Analytical Reagent grade.

Pesticides Studied:

Chloropyrifos, cypermethrin, dimethoate, endosulphan, fanvalerate and phosphamidon were procured from Bayer, India and used as such (**Table-2.1**).

Test Solutions:

All test solutions of pesticides were prepared in methanol.

Soil Samples:

We used five samples (S1-S5) of natural, uncultivated soils that were collected from the soil surface horizon (0-20cm deep) at different places in the district of Aligarh (India). The samples were dried, grounded and passed through the 100 mesh size sieve to get uniform particle size. The physical parameters of soil samples are given in **Table 2.2**

Detection:

Thin layer chromatography plates after development in a particular mobile phase were exposed to iodine vapours and the spot was visualized as dark brown/yellow in colour.

Stationary Phase:

Silica gel G, cellulose, kieselguhr, soil, soil + cellulose, soil + silica gel G, soil + kieselguhr (1:1, 7:3 and 3:7).

Mobile Phase:

Symbol	Composition	Symbol	Composition
M_1	Water	M_2	0.01M Ammonium sulphate
M_3	0.05 M Ammonium sulphate	M_4	0.10 M Ammonium sulphate
M_5	0.5 M Ammonium sulphate	M_6	1M Ammonium sulphate
M_7	1.5 M Ammonium sulphate	M_8	2.0 M Ammonium sulphate
M_9	0.01 M Sodium chloride	M_{10}	0.05 M Sodium chloride
M_{11}	0.10 M Sodium chloride	M_{12}	0.5 M Sodium chloride
M_{13}	1.0 M Sodium chloride	M_{14}	1.5 M Sodium chloride
M_{15}	2.0 M Sodium chloride	M_{16}	1% CTAB + 0.5 M Ammonium sulphate
M_{17}	1% CTAB in water	M_{18}	1% CTAB + 0.5 M sodium chloride
M_{19}	Methanol	M_{20}	Cyclohexane
M_{21}	Benzene	M_{22}	Hexane
M_{23}	Ethyl acetate	M_{24}	Toluene

CHROMATOGRAPHY

Preparation of Silica Gel TLC Plates

Plain Thin- Layer Plates

TLC plates were prepared by mixing silica gel G with double distilled water in a 1:3 ratio. The resultant slurry was mechanically shaken for 5 min and then it was coated onto glass plates with the help of a TLC applicator to give a layer of 0.25 mm thickness. The plates were first air dried at room temperature and then activated by heating at 100°C for 1 h. After activation, the plates were kept in air tight chamber until used. The TLC plates of cellulose, alumina and kieselguhr were similarly prepared.

Soil Thin-Layer Plates

To prepare soil TLC plates, soil sample was slurried mechanically for 5 min after mixing with double distilled water in a 1:3 ratio. The

resultant homogeneous slurry was spread onto 20 x 3.5 cm glass plates as 0.25 mm thick layer. The plates were air dried at room temperature (30^{o}C) and stored in air tight chamber until used.

Mixed Soil TLC Plates

Soil mixed with silica gel, kieselguhr, cellulose or alumina in different ratios (50:50, 70:30, 30:70) by weight were slurried with double distilled water in a 1:3 ratio by shaking for 5 min. Using this slurry, thin layers were prepared under the same experimental conditions as described above for soil thin layer plates.

Procedure

The pesticide solutions (5-10 µL) were spotted on TLC plates with micropipette. The spots were dried at room temperature before development. The glass jars containing mobile phase were covered with lids and left for 10 min for saturation before introducing the plates for development. The plates were developed with chosen solvent systems by ascending technique. The solvent ascent was fixed at 10 cm in all cases. After development, the plates were dried and the spots of pesticides were detected using iodine vapours.

Table – 2.1
IUPAC names and chemical formulae of pesticides used in the present study

Commercial name and abbreviation used	IUPAC name	Chemical formula
Chloropyrifos (CLPS)	0,0-diethyl 0-3,5,6 – trichloro -2-pyridyl phosphorothioate	$C_9H_{11}Cl_3NO_3PS$
Cypermethrin (CMN)	(Rs)-α-cyano-3-phenoxybenzyl (1Rs) - cis, trans-3-(2,2-dichloro-vinyl)-2,2 dimethyl-cyclo-propanecarboxycate	$C_{22}H_{19}Cl_2NO_3$
Dimethoate (DM)	0,0-dimethyl s-methyl carbamoylmethyl phosphorodithioate	$C_5H_{12}NO_3PS_2$
Endosulfan (ESN)	c,c-(1,4,5,6,7,7-hexachloro-8,9, 10-trinorborn-5-en-2,3-ylene) dimethyl sulphite	$C_9H_2Cl_6O_3S$
Fenvalerate (FVL)	(Rs)-α-cyano-3-phenoxybenzyl (Rs)-2-(4-chlorophenyl)-3-methyl butyrate	$C_{25}H_{22}Cl\,NO_3$
Phosphamidon (PHM)	2-chloro-2-diethylcarbamoyl-1-methyl vinyl dimethyl phosphate	$C_{10}H_{19}Cl\,NO_5P$

Data taken from : The agrochemicals handbook(Douglas Hastley and Hamish Kidd eds.), 2nd edition. royal society of chemistry (England) 1987.

2.3 RESULTS AND DISCUSSION

The results obtained have been summarized in **Tables 2.3-2.6**. The mobility (or R_F) data given in **Table 2.3** indicate that none of the pesticides migrates through the soil bed whereas PHM, CLPS and DM show significant mobility on silica layer when the plates were developed with distilled water. Thus, the soil has much stronger interactions to the pesticides compared to silica gel. To understand the interaction pattern of pesticides with different sorbent layers, several stationary and mobile phase systems were tested and the results obtained have been summarized in **Tables 2.4 a** and **b**.From the data listed in Table-2.4 following observations resulted in respect of mobility trend of pesticides through different static planar layers.

(A) Silica layer

(i) Aqueous Salt Solutions as Mobile Phases

When different concentrations of ammonium sulphate were used as mobile phase, pesticides such as ESN, CMN and FVL remained at the point of application at all concentration levels. The R_F value of PHM increased from 0.67 to 0.9 and that of CLPS decreased from 0.92 to 0.78 on increasing the concentration of ammonium sulphate from 0.01 to 2M in the mobile phase. A peculiar behaviour of DM was observed. It produced single spot at higher (1.5 – 2M) as well as at lower (0.01 - 0.05M) concentration of ammonium sulphate solution. However, it produced double spots with 0.1 or 1M ammonium sulphate and triple spots with 0.5M ammonium sulphate mobile phase. The multiple spots formation indicates the possibility of presence of different species of DM. With different concentrations of sodium chloride in the mobile phase ESN, CMN and FVL remained at the point of application. DM gave double spots over the entire concentration range (0.01–2M) of sodium chloride. PHM remained more or less at the middle of the chromatoplate ($R_F \cong 0.67$ -

0.42) whereas CLPS showed faster mobility ($R_F \cong 0.71 - 0.95$) at all sodium chloride concentration levels.

(ii) Aqueous Salt Solutions with Added Surfactant as Mobile Phases

When distilled water, 0.5 M aqueous ammonium sulphate or sodium chloride containing 1% added CTAB was used as the mobile phase, all pesticides remained at the point of application. Though all pesticides were clearly detected but the detection of PHM was difficult in the case of 0.5 M sodium chloride containing 1% CTAB.

(iii) Organic Solvent Mobile Phases

With benzene as the mobile phase, all pesticides did not show mobility. However, with other organic solvents like hexane and toluene CMN, FVL and ESN showed significant mobility ($R_F \cong 0.82, 0.87, 0.70$ respectively). This observation is important from the separation point of view because these pesticides were found to stay at their point of application with all the mobile phases mentioned above in (i) and (ii). Double spots for CLPS and DM were noticed with cyclohexane and methanol respectively. PHM showed irregular behaviour as it remained at the point of application in cyclohexane, benzene and hexane mobile phases; showed little tailing in toluene in ($R_F \cong 0.25$) and exhibited considerable mobility with methanol and ethyl acetate mobile phases ($R_F \cong 0.52, 0.70$ respectively). As regards to CLPS, it migrated along with most of the organic solvents ($R_F \cong 0.92$-0.94). Benzene and hexane are exceptions where CLPS remained very close to the point of application, ($R_F \cong 0.05$ and 0.18 respectively).

On the basis of migration behaviour of pesticides on silica layer as discussed above following interesting conclusions may be drawn:

(a) Hydrocarbon chain length of pesticides molecule does not influence the R_F, value as can be seen from the example of FVL and CMN. FVL ($C_{25}\ H_{22}\ Cl\ NO_3$) and CMN ($C_{22}\ H_{19}\ Cl_2\ NO_3$) showed identical

mobility and remained near or at the point of application when the TLC plates were developed with water or aqueous salt solutions (M_2-M_{15}).

(b) The presence of phosphorus in the pesticide molecule enhances the mobility. For example, PHM (C_{10} H_{19} Cl NO_5 P) showed higher mobility in water as well as in aqueous salt solutions (M_2–M_{15}) compared to CMN (C_{22} H_{19} Cl_2 NO_3) and FVL (C_{25} H_{22} Cl NO_3). The higher mobility of PHM may be attributed to its stronger complexation with the mobile phase. Furthermore, the higher mobility of P-containing PHM compared to S-containing ESN (Table 2.4a) is in support of the fact that phosphorus activates the mobility.

(c) Pesticides with higher P/S ratio have higher mobility as can be seen from the example of CLPS (C_9 H_{11} Cl_3 NO_3 PS) and DM (C_5 H_{12} NO_3 PS_2).CLPS showed higher mobility ($R_F \cong 0.92$) as compared to DM ($R_F \cong 0.67$) when water was used as mobile phase. With salt solutions as mobile phase (M_2-M_5), CLPS still showed greater mobility ($R_F \cong 0.71$ - 0.95) compared to DM ($R_F \cong 0.60$ - 0.85).

Thus, TLC can be used to distinguish between pesticides containing both P and S ligands in the same molecule.

(B) Soil Layer:

(i) Aqueous Salt Solution as Mobile Phases

Throughout the concentration of sodium chloride (0.05-2M), ESN, CMN, FVL were either not detected clearly in few cases or remained at the point of application in most of cases. PHM showed greater mobility ($R_F \cong$ 0.95) when 0.05 or 0.5 M sodium chloride was used as mobile phase. At higher sodium chloride concentrations, it could not be detected. Double spots for DM were realized when TLC plates were developed with 0.05 or 0.1 M aqueous sodium chloride. The R_F values were 0.0, 0.92 and 0.0,

0.87 respectively. CLPS showed mobility only when 0.10 M sodium chloride was taken as the mobile phase ($R_F \cong 0.92$).

With different concentrations of ammonium sulphate (0.01 - 2M) as mobile phase, PHM showed pronounced mobility at all concentration levels ($R_F \cong 0.75$ - 0.93). Similarly, higher mobility was also observed when CLPS ($R_F \cong 0.95$) and CMN ($R_F \cong 0.90$) were chromatographed using 1.0 M aqueous ammonium sulphate solution as mobile phase. DM was found to move with solvent front when aqueous ammonium sulphate (0.05 - 1.0 M) solutions were used as mobile phase. All other pesticides remained at the point of application irrespective of the degree of ammonium salt concentration in the mobile phase.

(ii) Aqueous Salt Solutions with Added Surfactant as Mobile Phases

When 0.5 M aqueous sodium or ammonium salt solutions containing 1% CTAB (M_{16} and M_{18}) was used as mobile phase, all pesticides remained at the point of application. PHM could not be detected clearly with M_{18}.

(iii) Organic Solvent Mobile Phases

The identical mobility pattern i.e. all pesticides remained at the point of application irrespective of the nature of organic mobile phase (M_{19} - M_{24}) was observed. PHM was not detected.

(C) Mixed Layers Containing Soil:

The results obtained on various mixed layers are briefly discussed below

(i) Soil Mixed with Cellulose

When soil mixed with cellulose (1:1, 7:3, 3:7) was used as the stationary phase in combination with water as mobile phase, all pesticides except PHM and ESN were well detected, and found to be at the point of application. With 0.5 M sodium chloride eluent, all pesticides stayed at the

point of application and PHM could not be detected. Interestingly, PHM showed high mobility ($R_F \cong 0.92$) when 0.5 M ammonium sulphate was taken as the mobile phase and soil mixed with cellulose in the ratio 3:7 was used as the stationary phase.

(ii) Soil Mixed with Silica gel Layer

For PHM, high mobilities were noticed on layers prepared from mixture of soil and silica in 7:3 ratio with water ($R_F \cong 0.8$) and 0.5 M sodium chloride ($R_F \cong 0.78$) eluents. Its detection on other mixed layers developed with water or aqueous salt solution was difficult. Double spots for DM were observed with certain TLC systems [water eluent, soil + silica gel layer (7:3); 0.5M sodium chloride eluent, soil + silica gel (7:3, 3:7, 1:1) layers, and 0.5 M ammonium sulphate eluent, soil + silica gel (3:7) layer]. ESN, CLPS, FVL and CMN were always found at the point of application.

(iii) Soil Mixed with Kieselguhr Layer

ESN, DM could not be detected on soil mixed with kieselguhr (1:1, 7:3, 3:7) layers. With water as mobile phase, PHM migrated with water front ($R_F \cong 0.90$) on layer of soil plus kieselguhr (7:3). Other pesticides remained at the point of application. When 0.5 M sodium chloride was used as the mobile phase, all but DM remained at the point of application. DM gave double spots on layers of soil plus kieselguhr, 1:1 ($R_F \cong 0.92$, 0.0) and 3:7 ($R_F \cong 0.87$, 0.0). Whereas it produced single spot ($R_F \cong 0.90$) on 7:3 layer. With 0.5 M ammonium sulphate eluent, none of the pesticides showed any mobility.

(D) Miscellaneous Layers:

To compare the retention efficiency of cellulose, kieselguhr and alumina as layer material, pesticides were chromatographed using 0.5 M sodium chloride and ammonium sulphate solutions as mobile phase. The

results obtained are summarized in Table 2.4a. Following retention pattern were noticed.

(a) With 0.5 m sodium chloride, the mobility of PHM increases in the order silica gel < kieselguhr <alumina <cellulose. CLPS moved with the solvent front ($R_F \simeq 0.9$) irrespective of the nature of stationary phase. An additional spot at the point of application was also observed for CLPS on kieselguhr layer. The R_F value of DM was in the order kieselguhr <silica gel < cellulose <alumina. It produces double spots on silica layer. The R_F of second spot was 0.0

(b) With 0.5 M ammonium sulphate, all pesticides have zero R_F value on alumina layer. The mobility trends of PHM and CLPS were in the order alumina < silica gel < kieselguhr < cellulose. In the case of DM, triple spots on silica gel; double spots on kieselguhr and single spot on cellulose were observed.

(c) With 0.5 M sodium chloride and ammonium sulphate, FVL, ESN and CMN remained at the point of application in all cases.

These results indicate that the multiplicity of pesticides spots depends upon the nature of stationary phase.

Table 2.5 summarizes the trend of relative mobility of a particular pesticide on different static layers (single phase as well as biphasic) developed with water, aqueous solution of (0.5 M) sodium chloride or ammonium sulphate. From this Table it is clear that the mobility of CLPS is higher on silica layer compared to soil or mixed layers in all mobile phases (M_1, M_5, M_{12}). Conversely, PHM and DM migrated faster through soil or soil mixed layers.

The separations obtained experimentally with different TLC systems are listed in **Table 2.6**. These separations are especially important because one can separate S-containing pesticides from those containing Cl, P or P and S both. Similarly, DM and CLPS containing S as well as P in their molecule can also be separated from their mixture.

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Table–2.2

pH and electrical conductivity data of different soil samples used as stationary phase

Soil Type	Place of collection	Texture	pH	EC(ohm^{-1})
S_1	A.M.U. Fort	Sandy Loam	8.30	0.874
S_2	Dhurrah Aligarh			
	(a) Sewage water Irrigated Soil	Sandy Loam	8.20	0.871
	(b) Tubewell Irrigated Soil	Sandy Loam	7.72	0.870
S_3	Tappal Soil	Clay	8.20	0.874
S_4	Jattari Soil	Loam	7.89	0.867
S_5	Botany Deptt. Soil. A.M.U., Aligarh	Sandy Loam	7.70	0.869

Table–2.3

Mobility trend of pesticides on silica gel and soil layer developed with water (M_1)

Pesticides	Silica gel (R_F)	Soil (R_F)
PHM	0.7	0.0
CLPS	0.92	0.0
ESN	0.0	0.0
CMN	0.0	0.0
FVL	0.07	0.0
DM	0.67	0.0

Table – 2.4 (a)

Mobility (R_F Value) of pesticides on single phase stationary phase using different mobile phases(M_2-M_{24})

Stationary phase	Mobile phase	Mobility (RF value)
Silica Gel	M_2	PHM (0.67), CLPS (0.92), DM (0.75),(ESN=CMN=FVL=0.0)
	M_3	PHM (0.62), CLPS (0.92), DM (0.85),(ESN=CMN=FVL=0.0)
	M_4	PHM (0.0.58),CLPS(0.87),DM (0.50,0.90), (ESN=CMN=FVL=0.0)
	M_5	PHM (0.57), CLPS (0.82), DM (0.82,0.45,0.0),(ESN=CMN=FVL=0.0)
	M_6	PHM (0.41), CLPS (0.72), DM (0.66,0.02), (ESN=CMN=FVL=0.0)
	M_7	PHM (0.79), CLPS (0.77), DM (0.78),(ESN=CMN=FVL=0.0)
	M_8	PHM (0.90), CLPS (0.78), DM (0.66), (ESN=CMN=FVL=0.0)
	M_9	PHM (0.80), CLPS (0.90,0.0), DM (0.87,0.0),(ESN=CMN=FVL=0.0)
	M_{10}	PHM (0.54), CLPS (0.90), DM (0.82,0.60), (ESN=CMN=FVL=0.0)
	M_{11}	PHM (0.67), CLPS (0.87), DM (0.60,0.0),(ESN=CMN=FVL=0.0)
	M_{12}	PHM (0.62), CLPS (0.92), DM (0.85,0.0),(ESN=CMN=FVL=0.0)
	M_{13}	PHM (0.52), CLPS (0.71), DM (0.80,0.0),(ESN=CMN=FVL=0.0)
	M_{14}	PHM (0.42), CLPS (0.93), DM (0.82,0.0),(ESN=CMN=FVL=0.0)
	M_{15}	PHM (0.42), CLPS (0.95), DM (0.80,0.0), (ESN=CMN=FVL=0.0)
	M_{16}	PHM =CLPS =DM =ESN=CMN=FVL=0.0
	M_{17}	PHM =CLPS =DM =ESN=CMN=FVL=0.0
	M_{18}	PHM (ND), CLPS =DM=ESN=CMN=FVL=0.0
	M_{19}	PHM (0.52), CLPS (0.92), DM (0.90,0.0)ESN=CMN=FVL=0.0
	M_{20}	CLPS (0.16,0.0),PHM=DM ESN=CMN=FVL=0.0
	M_{21}	PHM =CLPS =DM ESN=CMN=FVL=0.0
	M_{22}	CLPS (0.18), ESN (0.70),CMN (0.25), FVL (0.25), (PHM = DM=0.0)
	M_{23}	PHM (0.70), CLPS (0.95), DM (0.50),(ESN=CMN=FVL=0.0)
	M_{24}	PHM (0.25), CLPS (0.92), CMN(0.87),FVL(0.87), (ESN =CMN=0.0)
Soil	M_{10}	PHM (0.95), DM(0.92,0.0),ESN(ND)(ESN=CMN=FVL=0.0)

Continued

Stationary phase	Mobile phase		Mobility (RF Value)
	M_{11}	PHM	(ND),CLPS(0.92),DM(0.87,0.0), (ESN=CMN=FVL=0.0)
	M_{12}	PHM	(0.95),(CLPS=ESN=DM = CMN = FVL=0.0)
	M_{13}	PHM	(ND), ESN(ND),(CLPS=DM=CMN=FVL=0.0)
	M_{14}	PHM	(ND),(ESN=CLPS=DM=CMN=FVL=0.0)
	M_{15}	PHM	= ESN=CLPS =DM=CMN=FVL=0.0
	M_2	PHM	(0.92) (CLPS = ESN = CMN = FVL = DM =0.0)
	M_3	PHM	(0.75) (CLPS = ESN = CMN = FVL = DM =0.0)
	M_4	PHM	(0.90) (CLPS = ESN = CMN = FVL = DM =0.0)
	M_5	PHM	(0.87), CLPS (0.95),DM (0.87), CMN (0.90) (ESN=FVL=0.0)
	M_6	PHM	(0.93), DM (0.92) (CLPS = ESN = CMN = FVL = 0.0)
	M_7	PHM	(0.85), (CLPS= ESN=CMN=FVL=DM=0.0)
	M_8	PHM	(0.90), (CLPS=ESN=CMN=FVL=DM=0.0)
	M_{16}	PHM	(ND), (CLPS=ESN=CMN=FVL=DM=0.0)
	M_{17}	PHM	(ND), (CLPS=ESN=CMN=FVL=DM=0.0)
	M_{18}	PHM	(ND), (CLPS=ESN=CMN=FVL=DM=0.0)
	M_{19}	PHM	(ND), (CLPS=ESN=CMN=FVL=DM=0.0)
	M_{20}	PHM	(ND), (CLPS=ESN=CMN=FVL=DM=0.0)
	M_{21}	PHM	(ND), (CLPS=ESN=CMN=FVL=DM=0.0)
	M_{22}	PHM	(ND), (CLPS=ESN=CMN=FVL=DM=0.0)
	M_{23}	PHM	(ND), (CLPS=ESN=CMN=FVL=DM=0.0)
	M_{24}	PHM	(ND), (CLPS=ESN=CMN=FVL=DM=0.0)
Cellulose	M_5	PHM	(0.90),CLPS (0.97),DM (0.91), FVL (0.05) (ESN=CMN=0.0)
	M_{12}	PHM	(0.95),CLPS (0.95), DM (0.90),(ESN=CMN=FVL=0.0)
Kieselguhr	M_5	PHM	(0.87),CLPS(0.87),FVL(0.05), DM (0.77,0.0) (ESN=CMN=0.0)
	M_{12}	PHM	(0.82),CLPS(0.62,0.90),FVL(0.10), DM (0.75) (ESN=CMN=0.0)
Alumina	M_5	PHM	(0.0), (CLPS=ESN=CMN=FVL=DM=0.0)
	M12	PHM	(0.88),CLPS(0.95),DM (0.92), (ESN=CMN=FVL=0.0)

Table – 2.4 (b)

R_F value of pesticides on biphasic stationary phase using mobile phases M_1, M_5 and M_{12}

Stationary	Mobile phase	R_F Value
Soil + Cellulose		
(1:1)	M_1	(PHM=ESN=ND), (CLPS=DM=FVL=CMN=0.0)
(3:7)	M_1	PHM (ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(7:3)	M_1	PHM (ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(1:1)	M_{12}	PHM (ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(3:7)	M_{12}	PHM (ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(7:3)	M_{12}	PHM (ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(1:1)	M_5	PHM (ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(3:7)	M_5	PHM (0.92), (ESN=CLPS=DM-FVL=CMN=0.0)
(7:3)	M_5	PHM (ND), (ESN=CLPS=DM-FVL=CMN=0.0)
Soil + silica gel		
(1:1)	M_1	PHM (ND), (ESN=CLPS=DM-FVL=CMN=0.0)
(3:7)	M_1	PHM(ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(7:3)	M_1	PHM(0.85), DM (0.82,0.0), (ESN=CLPS=FVL=CMN=0.0)
(1:1)	M_{12}	DM(0.85,0.0), (ESN=CLPS=DM=FVL=CMN=0.0)
(3:7)	M_{12}	DM(0.80, 0.0) , PHM(ND), (ESN-CLPS=FVL=CMN=0.0)
(7:3)	M_{12}	PHM (0.78), DM (0.87,0.0) (ESN=CLPS=FVL=CMN=0.0)
(1:1)	M_5	PHM(ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(3:7)	M_5	DM(0.80,0.0), PHM(ND), (ESN=CLPS=FVL=CMN=0.0)
(7:3)	M_5	PHM(ND), (ESN=CLPS=DM=FVL=CMN=0.0)
Soil + kieselguhr		
(1:1)	M_1	PHM(ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(3:7)	M_1	(PHM=DM=ND), (ESN=CLPS=FVL=CMN=0.0)
(7:3)	M_1	(ESN=DM=ND), PHM(0.90), (CLPS=FVL=CMN=0.0)
(1:1)	M_{12}	DM(0.92,00),PHM(ND),(ESN=CLPS=FVL=CMN=0.0)
(3:7)	M_{12}	DM(0.87,0.0),PHM(ND),(ESN=CLPS=FVL=CMN=0.0)
(7:3)	M_{12}	DM (0.90), PHM(ND), (ESN=CLPS=FVL=CMN=0.0)
(1:1)	M_5	PHM (ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(3:7)	M_5	PHM (ND), (ESN=CLPS=DM=FVL=CMN=0.0)
(7:3)	M_5	PHM (ND), (ESN=CLPS=DM=FVL=CMN=0.0)

Table – 2.5

Mobility trend of pesticides on various stationary phases used

Mobile phase	CLPS	PHM[a]	DM
M_1	Silica gel> soil = soil + silica gel (3:7) = soil + kieselguhr(3:7) = soil + cellulose (3:7)	Soil + kieselguhr > soil + silica gel> silica gel > soil	Soil + silica gel > silica gel > soil + kieselguhr = soil + cellulose = soil
M_5	Silica gel> soil = soil + silica gel = soil + kieselguhr = soil + cellulose	Soil > soil + cellulose > silica gel	Soil > silica gel [b]> soil + silica gel [c]> soil + kieselguhr = soil + cellulose
M_{12}	Silica gel > soil = soil + kieselguhr = soil + silica gel = soil + cellulose	Soil > silica gel	Soil + kieselguhr [c]> silica gel [c]> soil + silica gel [c] > soil = soil + cellulose

a) PHM could not be detected on other stationary phase
b) Triple spots (RF 0.82, 0.45 and 0.0), RF 0.82 is taken for comprison
c) Double spots (second spot at RF = 0.0 in all cases). Higher RF value is taken for comparison ESN, CMN and FVL remain at or near the point of application on all stationary phases.

Table–2.6

Separtions achieved experimentally on silica gel, soil and amended soil layers

Stationary phase	Mobile phase	Separation (R_F)
Silica gel	M_2	DM (0.72) - ESN, CMN or FVL (0.0)
		CLPS (0.92) - ESN, CMN or FVL (0.0)
		PHM (0.67) - ESN, CMN or FVL (0.10)
	M_{19}	PHM (0.52) - ESN, CMN or FVL (0.0)
		CLPS (0.92) - ESN, CMN or FVL (0.0)
	M_{22}	ESN (0.70) - PHM, DM, CMN or FVL (0.25)
	M_9	DM (0.82) - CMN or FVL (0.0)
		CLPS (0.90) - ESN, CMN or FVL (0.10)
		PHM (0.75) - ESN, CMN or FVL (0.0)
Soil	M_2	DM (0.90) - CMN, CLPS, ESN or FVL (0.0)
		PHM (0.90) - CMN, CLPS, ESN or FVL (0.0)
	M_9	DM (0.85) - CMN, CLPS, ESN or FVL (0.0)
		PHM (0.87) - CMN, CLPS, ESN or FVL (0.0)
Soil + silica gel (7:3)	M_1	PHM (0.85) - CLPS, ESN, CMN or FVL (0.0)
Soil + silica gel (7:3)	M_{12}	PHM (0.78) - CLPS, ESN, CMN or FVL (0.0)
Soil + kieselghur (7:3)	M_1	PHM (0.90) - CLPS, CMN or FVL (0.0)
Soil + cellulose (3:7)	M_5	PHM (0.92) CLPS, ESN, CMN, FVL or DM (0.0)

CHAPTER-III

TLC studies and separation of heavy metal cations on soil amended silica gel layers developed with surfactant-mediated solvent systems.

3.1 INTRODUCTION

Water pollution due to heavy metal pollutants cause direct toxicity, both to humans and other living beings, due to their presence beyond specified limits. Thin layer chromatography (TLC) has made a major contribution to the analysis of inorganic cations and being low cost technique it is still enjoying the popularity as an undisputed analytical tool. The recent applications of TLC have been well documented in reviews (1-4) books (5-7) and research papers (8-12) .

A new concept for qualitative analysis by soil TLC was developed by Helling and Turner in 1968 (13). Their method was utilized by several workers to examine the mobility pattern of pesticides (14-17) and heavy metals (18-23) in various types of soils. The mobility or leachability of such chemicals through the soil bed has tremendous influence on the life process of plants. An interesting study on mobility of cadmium on twenty-two soil layers of different nature has been reported by M. Sanchez Camazano and M.J. Sanchez-Martin (24). Their results clearly demonstrate the significant influence of soil properties on the mobility of cadmium.

TLC methods reported so far for investigating the mobility of heavy metals and trace elements in soil did not consider the following two aspects.

(i) Use of surfactants as mobile phase.

(ii) Effect of presence of silica, alumina; cellulose and kieselguhr in soil bed on the mobility of heavy metal cations.

In view, of the above facts, it was considered worthwhile to examine the mobility of heavy metal cations through static planar soil bed amended with silica, alumina, cellulose and kieselguhr using aqueous solutions of cationic, anonic and non ionic surfactants as mobile phase. The results of this study may be of immense utility to understand the role of surfactant

mediated mobile phases on the mobility or leachability of heavy metals through pure and soil amended layers.

3.2 EXPERIMENTAL

Chemicals and Reagents:

Silica gel G (Merck, India), N-cetyl-N, N, N- trimethyl ammonium bromide (CTAB) of CDH India, urea (GSC, India), sodium dodecyl sulphate (SDS) of BDH India. Polyoxyethylene dodecyl ether (Brij35) and iso-octylphenoxypolyethoxy-ethanol (TX-100) of Loba Chemie, India were used.

Metal Ions Studied:

Pb^{2+}, Bi^{3+}, Zn^{2+}, Ag^{+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Hg^{2+}, Cd^{2+} and Fe^{3+}

Test Solution:

Chromatography was performed on 1% standard solutions of the chloride, nitrate or sulphate salts of the above mentioned metal ions.

Soil Sample:

Samples (S_1-S_8) used were collected from different locations in the district of Aligarh (India) the method of collection is similar to what has been mentioned in Chapter II. The physico-chemical parameters of soil samples are given in **Table 3.6**

Detection:

Fe^{3+}, Cu^{2+} were detected with 1% potassium ferrocyanide; Ni^{2+} and Co^{2+} were detected with 1% solution of alcoholic dimethylglyoxime and Zn^{2+}, Cd^{2+} Pb^{2+}, Bi^{3+}, Ag^{+} and Hg^{2+} were detected with a solution of 0.5% dithizone in carbon tetra chloride.

Mobile Phase:

Solvent System	Symbol	Composition
Aqueous surfactant solution	M_1	0.1 M CTAB
	M_2	0.1 M SDS
	M_3	0.1 M TX-100
	M_4	0.1 M Brij-35
	M_5	0.01 M CTAB
	M_6	0.001 M CTAB
	M_7	0.0001 M CTAB
	M_8	0.3 M CTAB
	M_9	0.4 M CTAB
	M_{10}	0.5 M CTAB
Fertilizer added surfactant solution	M_{11}	0.5 M CTAB + 0.1 M urea (1:1)
	M_{12}	0.5 M CTAB + 0.1 M urea (1:9)
	M_{13}	0.5 M CTAB + 0.1 M urea (9:1)
	M_{14}	0.5 M CTAB + 0.6 M urea (1:9)
	M_{15}	0.5 M CTAB + 0.6 M urea (1:1)
	M_{16}	0.5 M CTAB + 0.6 M urea (9:1)
	M_{17}	0.5 M CTAB + 0.8 M urea (1:1)
	M_{18}	0.5 M CTAB + 0.8 M urea (1:9)
	M_{19}	0.5 M CTAB + 0.8 M urea (9:1)
	M_{20}	0.5 M CTAB + 2 M urea (1:1)
	M_{21}	0.5 M CTAB + 2 M urea (9:1)
	M_{22}	0.5 M CTAB + 2 M urea (1:9)
	M_{23}	0.5 M CTAB + 0.6 M ammonium chloride (1:1)
	M_{24}	0.5 M CTAB + 0.6 M ammonium nitrate (1:1)
	M_{25}	0.5 M CTAB + 0.6 M sodium phosphate (1:1)

Mixed mobile phases were prepared by mixing different volumes of individual solution/solvent.

Stationary Phase:

Symbol	Composition
(A)	Pure silica gel
(B)	Pure soil
S_1 -	Soil botany deptt.
S_2 -	Soil A.M.U. fort
S_3 -	Dhurrah sewage irrigated soil (15 cm depth)
S_4 -	Dhurrah sewage irrigated soil (20 cm depth)
S_5 -	Dhurrah tubewell irrigated soil (15 cm depth)
S_6 -	Dhurrah tubewell irrigated soil (20 cm)
S_7 -	Jattari soil
S_8 -	Tappal soil
(C)	
S_9 -	Silica gel mixed with soil type S_1 (9:1)
S_{10} -	Silica gel mixed with soil type S_2 (9:1)
S_{11} -	Silica gel mixed with soil type S_3 (9:1)
S_{12} -	Silica gel mixed with soil type S_4 (9:1)
S_{13} -	Silica gel mixed with soil type S_5 (9:1)
S_{14} -	Silica gel mixed with soil type S_6 (9:1)
S_{15} -	Silica gel mixed with soil type S_7 (9:1)
S_{16} -	Silica gel mixed with soil type S_8 (9:1)
(D) -	Silica gel mixed with soil type S_3 in the ratios (8:2), (7:3), (6:4), (5:5), (4:6), (3:7), (2:8), (1:9)
(E)	Alumina + soil type (S_3) 9:1
	Cellulose + soil type (S_3) 9 :1
	Kieselguhr + soil type (S_3) 9:1, flyash + soil type (S_3) 9:1

CHROMATOGRAPHY

Preparation of Silica Gel TLC Plates

Plain Thin – Layer Plates

For the preparation of plain thin–layer plates similar procedure was followed as described in chapter II.

Soil Thin – Layer Plates

Soil TLC plates were prepared as mentioned in chapter II.

Mixed Soil TLC Plates

Silica gel, kieselguhr, cellulose or alumina mixed with soil in 9:1 ratio by weight. The contents were slurred with double distilled water in a 1:3 ratio by shaking for 5 min. Using this slurry, thin layers were prepared under the same experimental conditions as described above for soil layer plates.

Procedure

The metal ion solutions (5-10 *u*L) were spotted on TLC plates with micropipette. The spots were dried at room temperature before development. The glass jars containing mobile phase were covered with lids and left for 10 min. for saturation before introducing the plates for development. The plates were developed with chosen solvent system by ascending technique. The solvent ascent was fixed at 10 cm in all cases. After development, the plates were dried and the spots of metal ions were detected using appropriate reagent.

The reproducibility of R_F values on S_{11} developed with M_{15} was checked by three independent analyses and by the same analyst on different days under identical experimental conditions, in the same laboratory, using the same apparatus.

3.3 RESULTS AND DISCUSSION

The results presented in **Table 3.1** reveal that the mobility of metal cations is decreased with the increase of soil concentration in the stationary phase. The soil concentration above 50% in the stationary phase was undesirable because of (a) lack of differential migration as all metal ions are strongly retained by the stationary phase near the point of application, (b) formation of tailed spots in certain cases, and (c) poor detection of Cu^{2+} and Co^{2+}.

The best results, with reasonably good detection clarity, spot compactness and differential migration of metal cations were realized on layers prepared from a mixture consisting of silica gel and soil type S_3 in 90:1 ratio (termed as S_{11} in this paper). Therefore, it was selected for detailed study. It is also evident from the results recorded in Table 3.1 that amongst the surfactants used, 0.1 M aqueous CTAB (M_1) is a useful eluent to achieve important separations of metal ions. With this system, Hg^{2+} showed maximum R_F value ($R_F \sim 0.96$) and Cd^{2+} moved nearly up to the middle of the chromatoplate ($R_F = 0.41$). Both the metal ions remained more or less at the point of application when 0.1 M aqueous solutions of non-ionic surfactants (M_3 & M_4) were used as the mobile phase. Similarly, Pb^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Bi^{3+} and Ag^{+} showed little mobility in aqueous surfactant solutions irrespective of the nature of the hydrophilic group of the surfactant (M_1-M_4).

It is important to mention here that none of the metal ions tends to exhibit mobility in the absence of surfactant as all the metal ions were found to remain at the point of application ($R_F = 0.0$) when chromatographed on soil layer (S_3) using distilled water (zero surfactant) as mobile phase.

The results of TLC of metal ions performed on selected stationary phase (S_{11}) using different CTAB concentrations (M_5-M_{10}) as mobile phase are listed in **Table 3.2**. The results reported in Table 3.2 indicate that the mobility of Zn^{2+}, Cd^{2+} and Hg^{2+} increased with the increase in the

concentration of CTAB from 0.001 to 0.5 M. Interestingly, the clarity of spot detection was also found to be associated with the degree of concentration of CTAB. The heavy metal cations were detected with enhanced clarity when 0.5 M CTAB was used as eluent. The increased mobility of certain cations facilitates the separation of Cd^{2+} from Zn^{2+} and Hg^{2+}. Preparation of CTAB solutions with higher concentrations exceeding to 0.5 M was difficult because of solubility limitations and hence no further studies with higher concentration of CTAB could be performed. Fe^{3+}, Cu^{2+}, Ag^{+}, Pb^{2+} and Bi^{3+} remained at or near the point of application at all concentration levels. Ni^{2+} and Co^{2+} showed little mobility (R_F = 0.41) and gave almost identical results over the entire CTAB concentration range.

Different concentrations of urea (M_{11}-M_{22}) were added in 0.5 M CTAB in variable ratios and the results of mobility of metal ions with urea added surfactant mobile phases using S_{11} layer are tabulated in **Table 3.3**. Amongst urea added mobile phases, the mobile phase consisting of 0.5 M CTAB plus 0.6 M urea in 1:1 ratio (M_{15}) was found most useful. At this concentration level, Cu^{2+} showed slight mobility (R_F = 0.38) to facilitate its separation from Ni^{2+}, Pb^{2+}, Hg^{2+} and Fe^{3+}. It was found that when 0.1 M urea solution was added into 0.5 M CTAB in different volume ratio (M_{11}-M_{13}), Cu^{2+} remained near the point of application (R_F = 0.07-0.18). Similarly, with mobile phases such as M_{14}, M_{18} and M_{22} Cu^{2+} was strongly retained by the stationary phase giving R_F value 0.10, 0.12 and 0.08 respectively. At all concentration levels of urea in the mobile phase, metal ions such as Fe^{3+}, Zn^{2+}, Pb^{2+}, Bi^{3+} remained at the point of application or showed very little mobility whereas Ni^{2+}, Co^{2+}, Cd^{2+} and Hg^{2+} showed considerable mobility. As better separation possibilities were dictated by M_{15} it was selected for detailed study. At first instance, we investigated the mobility behaviour of metal cations on TLC plates coated with pure different types of soil (S_1-S_8) using M_{15} as mobile phase. None of the cations show any mobility on pure soil layers and remained at the point application (R_F = 0.05). The high pH value of the soil samples (pH > 7.0)

seems to be responsible for the low mobility of cations. These results are in consonance with the observations of Sanchez (24), who have reported low mobility ($R_F = 0.14$) of Cd^{2+} in soils whose pH values is ≥ 6.3. Most of the metal ions were either not detected or remained at or near the point of application. Hg^{2+} was the exception, which migrates on S_3 and S_4 layers showing R_F value of 0.62. However, Hg^{2+} could not be clearly detected on layers prepared from S_1, S_2 and S_5-S_8.

In order to examine the effect of inorganic fertilizers on the mobility of metal ions, urea (organic fertilizer) was replaced by three other fertilizers (ammonium chloride, ammonium nitrate and sodium phosphate) in aqueous CTAB mobile phase and TLC of metal ions was performed using different soil types (S_1-S_8) mixed with silica gel in 1:9 ratio as the stationary phase (S_9-S_{16}). The results summarized in **Table 3.4** show the following trends.

(i) Detection of metal ions was not sharp when sodium phosphate was used in the mobile phase whereas such a problem was not observed with mobile phases containing ammonium nitrate or chloride. In these cases detection of metal ions was sharp and spots were highly compact.

(ii) Co^{2+}, Ni^{2+}, Hg^{2+} and Bi^{3+} gave comparable results (i.e. almost identical mobility) irrespective of the type of fertilizer present in the mobile phase. Bi^{3+} remained near the point of application or showed very little mobility while Ni^{2+}; Co^{2+} and Hg^{2+} moved with the solvent front (M_{15}, M_{23}, M_{24}, M_{25}) on layers prepared from (S_9-S_{16}).

(iii) With sodium phosphate added aqueous CTAB mobile phase (M_{25}), Cu^{2+} showed stronger interaction with the stationary phase giving the R_F value of 0.03 irrespective of the type of soil used in the stationary phase. However, and enhanced mobility ($R_F \cong 0.25$-0.38) was experienced when other fertilizers such as NH_4NO_3, NH_4Cl and

urea were taken in the mobile phase (M_{24}, M_{23} or M_{15}). It shows that the mobility of Cu^{2+} is influenced by the nature of fertilizer.

(iv) In contrast to the behavior of Cu^{2+}, Zn^{2+} which remained at or near the point of application in all fertilizers containing CTAB mobile phases showed an increased mobility ($R_F \cong 0.3$-0.57) when sodium phosphate was present in the mobile phase.

(v) Cd^{2+} showed peculiar behavior with sodium phosphate as it gave double spots ($R_F \cong 0.14$-0.22 1^{st} spot, $R_F \cong 0.69 - 0.77$ 2^{nd} spot) in all soil types used in the stationary phase with the exception of S_{11} and S_{16} layers where it gave elongated single ($R_F \cong 0.49$) and compact single ($R_F \cong 0.69$) spots respectively. The formation of double spots shows the presence of two species. It seems that the lower spot is due to Cd- phosphate complex whereas the upper spot is due to the free Cd^{2+} ions.

Separations achieved on different types of soil containing layers using M_{15} mobile phase are listed in **Table 3.5**. In most of the cases Cd^{2+} was easily separated from Zn^{2+} and Hg^{2+}. Quaternary separations were also achieved on almost all soil mixed with silica gel layers. Thus, we can safely conclude that urea added aqueous cationic surfactant solutions are very useful mobile phases for separating heavy metal cations from their multicomponent mixtures. Most of the separations achieved were on soil containing silica mixed layers. The type of soil used in combination with silica influences the mobility as well as the detection clarity of metal cations. The soil mixed silica stationary phases can be used in the following order of preference to realize selected separation of metal cations

$$S_{11}=S_{13}=S_{15} > S_9=S_{12}>S_{14}>S_{16}>S_{10}$$

Since the mobility of metal cation was found to depend on the degree of silica gel present in the mixed bed containing soil, the results obtained have been summarized in **Fig. 3.1**. The following trends are noticeable:

analyses and by the same analyst in different days did not differ by more than 0.15 (i.e. ±15%) from the average R_F value, indicating a good reproducibility.

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Table – 3.1

R_F value (mobility trend)[a] of heavy metal cations on pure silica gel and soil (type S_3) plus silica gel mixed layers developed with aqueous solutions of different type of surfactants.

Metal ions	M_1				M_2				M_3				M_4			
	Pure SG	SG+Soil 90:10	SG+ Soil 70:30	SG+ Soil 50:50	Pure SG	SG+Soil 90:10	SG+Soil 70:30	SG+ Soil 50:50	Pure SG	SG+Soil 90:10	SG+Soil 70:30	SG+ Soil 50:50	Pure SG	SG+Soil 90:10	SG+Soil 70:30	SG+ Soil 50:50
Fe^{3+}	0.05	0.09	0.04	0.04	0.05	0.09	0.04	0.04	0.05	0.09	0.04	0.04	0.05	0.09	0.04	0.04
Co^{2+}	0.46	0.56	0.30	ND	0.73	0.53	0.37	ND	0.35	0.57	0.21	ND	0.40	0 56	0.24	ND
Ni^{2+}	0.60	0.56	0.35	0.17	0.90	0.52	0.46	0.21	0.45	0.56	0.22	0.13	0.43	0.55	0.27	0.12
Cu^{2+}	0.08	0.10	0.03	0.02	0.05	0.05	0.03	0.10	0.02	0.06	0.01	0.02	0.04	0.05	0.02	0.01
Zn^{2+}	0.10	0.14	0.09	0.05	0.08	0.18	0.06	0.06	0.05	0.23	0.05	0.04	0.06	0.11	0.05	0.03
Ag^{+}	0.0	0.0	0.0	0.0	0.08	0.14	0.07	0.05	0.23	0.18	0.13	0.11	0.28	0.24	0.18	0.08
Cd^{2+}	0.40	0.41	0.32	0.22	0.42	0.4	0.22	0.15	0.17	0.20	0.15	0.08	0.21	0.26	0.12	0.08
Hg^{2+}	0.97	0.96	0.96	0.97	0.35	0.27	0.13	0.10	0.17	0.22	0.09	0.05	0.12	0.21	0.07	0.05
Pb^{2+}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bi^{3+}	0.16	0.26	0.12	0.11	0.12	0.15	0.12	0.25	0.20	0.2	0.14	0.12	0.17	0.16	0.15	0.15

a) Mobility of all metal ions on pure soil layer was minimal ($R_F \cong 0.05$).
ND refers to not detected

Table – 3.2

R_F Value of metal Ions on S_{11} layer developed with various concentrations of CTAB

Metal ion	M_5	M_6	M_7	M_8	M_9	M_{10}
Fe^{3+}	0.05	0.06	0.05	0.05	0.06	0.07
Co^{2+}	0.49	0.51	0.59	0.58	0.62	0.55
Ni^{2+}	0.52	0.52	0.57	0.56	0.60	0.57
Cu^{2+}	0.06	0.06	0.06	0.06	0.05	0.07
Zn^{2+}	0.12	0.10	0.13	0.16	0.15	0.23
Ag^{+}	0.12	0.40	0.37	0.15	0.14	0.13
Cd^{2+}	0.24	0.25	0.22	0.17	0.57	0.64
Hg^{2+}	0.87	0.29	0.0	0.78	0.80	0.81
Pb^{2+}	0.0	0.0	0.0	0.0	0.0	0.0
Bi^{3+}	0.16	0.20	0.22	0.15	0.15	0.2

Table – 3.3

R_F Value of metal ions on S_{11} developed with 0.5 M aqueous CTAB containing different concentrations of added urea

Metal ions	M_{11}	M_{12}	M_{13}	M_{14}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{20}	M_{21}	M_{22}
Fe^{3+}	0.07	0.06	0.06	0.08	0.08	0.07	0.08	0.1	0.06	0.06	0.05	0.07
Co^{2+}	0.59	0.56	0.59	0.70	0.75	0.63	0.76	0.7	0.62	0.63	0.64	0.66
Ni^{2+}	0.64	0.61	0.57	0.70	0.7	0.67	0.69	0.68	0.67	0.65	0.68	0.7
Cu^{2+}	0.18	0.07	0.07	0.10	0.38	0.38	0.31	0.12	0.3	0.35	0.33	0.08
Zn^{2+}	0.19	0.1	0.17	0.11	0.22	0.19	0.13	0.12	0.16	0.15	0.19	0.1
Ag^{+}	0.11	0.06	0.17	0.06	0.13	0.15	0.15	0.1	0.2	0.12	0.12	0.6
Cd^{2+}	0.17	0.51	0.61	0.55	0.74	0.62	0.64	0.60	0.70	0.67	0.65	0.52
Hg^{2+}	0.13	0.87	0.96	0.92	0.96	0.87	0.96	0.96	0.93	0.97	0.92	0.96
Pb^{2+}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bi^{3+}	0.17	0.22	0.15	0.19	0.24	0.20	0.2	0.22	0.17	0.22	0.17	0.21

Table – 3.4

R_F Value of metal ions obtained on TLC plates prepared of silica gel mixed with different types of soils in 9:1 ratio (S_9-S_{16}) developed with 0.5 M CTAB with added fertilizer.

Mobile Phase	Metal Ions	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}
M_{15}		0.55	0.52	0.75	0.72	0.42	0.68	0.7	0.6
M_{24}	Co^{2+}	0.78	0.76	0.66	0.76	0.81	0.75	0.85	0.64
M_{25}		0.73	0.87	0.73	0.82	0.79	0.84	0.68	0.71
M_{23}		0.87	0.79	0.82	0.9	0.84	0.51	0.89	0.82
M_{15}		0.64	0.54	0.74	0.67	0.49	0.66	0.78	0.50
M_{24}	Ni^{2+}	0.78	0.77	0.70	0.77	0.77	0.74	0.87	0.65
M_{25}		0.79	0.87	0.76	0.87	0.83	0.85	0.70	0.73
M_{23}		0.86	0.82	0.85	0.89	0.89	0.50	0.89	0.85
M_{15}		0.31	ND	0.38	0.34	0.2	0.31	0.34	ND
M_{24}	Cu^{2+}	0.26	0.19	0.19	0.23	0.23	0.26	0.24	0.21
M_{25}		0.03	0.03	0.03	0.02	0.02	0.02	0.03	0.02
M_{23}		0.33	0.3	0.23	0.37	0.35	0.25	0.33	0.27
M_{15}		0.12	0.10	0.22	0.23	0.05	0.14	0.17	0.11
M_{24}	Zn^{2+}	0.17	0.12	0.14	0.12	0.16	0.14	0.13	0.14
M_{25}		0.57	0.55	0.3	0.52	0.41	0.45	0.6	0.44
M_{23}		0.14	0.13	0.17	0.17	0.28	0.12	0.25	0.10
M_{15}		0.65	0.50	0.74	0.72	0.36	0.65	0.63	0.47
M_{24}		0.76	0.78	0.71	0.75	0.77	0.82	0.76	0.7
M_{25}	Cd^{2+}	0.72, 0.22	0.77, 0.20	0.49T	0.74, 0.18	0.69, 0.16	0.77, 0.14	0.74, 0.20	0.69
M_{23}		0.75	0.72	0.72	0.85	0.81	0.4	0.82	0.78
M_{15}		0.92	0.93	0.96	0.99	0.80	0.97	0.93	0.87
M_{24}	Hg^{2+}	0.92	0.93	0.87	0.87	0.88	0.96	0.93	0.92
M_{25}		0.88	0.81	0.96	0.87	0.96	0.92	0.96	0.89
M_{23}		0.93	0.80	0.91	0.97	0.88	0.57	0.9	0.95
M_{15}		0.21	0.17	0.24	0.18	0.17	0.22	0.23	0.15
M_{24}	Bi^{3+}	0.19	0.14	0.15	0.25	0.36	0.23	0.22	0.14
M_{25}		0.12	0.10	0.15	0.16	0.14	0.16	0.12	0.17
M_{23}		0.17	0.12	0.15	0.35	0.38	0.22	0.24	0.25

The variation in R_F values of Fe^{3+}, Ag^{+} and Pb^{2+} was found to be in the range of 0.05-0.11, 0.08-0.13 and 0.0-0.05 respectively depending upon the type of soil (S_1-S_8) used.

T refers to tailed spot (R_L - R_T >0.3)

ND refers to not detected

Table – 3.5

Experimentally achieved separations of metal ions on soil mixed with silica gel layers developed with mobile phase 0.5 M CTAB + 0.6 M Urea (1:1).

Stationary phase	Separations (R_F)
S_9	Ni^{2+} (0.65) – Cu^{2+} (0.30) – Fe^{3+} (0.05), Ni^{2+} (0.62) – Cu^{2+} (0.3) – Pb^{2+} (0.0), Ni^{2+} (0.65) – Cu^{2+} (0.3) – Fe^{3+} (0.0) – Hg^{2+} (0.99), Zn^{2+} (0.12) – Cd^{2+} (0.6)- Hg^{2+} (0.95), Zn^{2+} (0.18)- Fe^{3+} (0.0) – Ni^{2+} (0.62) – Hg^{2+} (0.92).
S_{10}	Zn^{2+} (0.12)–Cd^{2+} (0.55)–Hg^{2+} (0.95), Ni^{2+} (0.7)–Fe^{3+} (0.03)–Hg^{2+} (0.94).
S_{11}	Ni^{2+} (0.72) – Cu^{2+} (0.21) – Fe^{3+} (0.05), Ni^{2+} (0.70) – Cu^{2+} (0.26) – Pb^{2-}(0.0), Fe^{3+} (0.02) – Cu^{2+} (0.24) – Ni^{2+} (0.68) – Hg^{2+} (0.97), Zn^{2+} (0.11) – Cd^{2+} (0.67) – Hg^{2+} (0.95), Zn^{2+} (0.17) – Fe^{3+} (0.0) – Ni^{2+} (0.63) – Hg^{2+} (0.95), Pb^{2+} (0.0)- Cd^{2+} (0.7) – Hg^{2+} (0.96).
S_{12}	Ni^{2+} (0.61) – Cu^{2+} (0.24) – Fe^{3+} (0.3), Fe^{3+} (0.02) – Cu^{2+} (0.31) – Hg^{2+} (0.99), Ni^{2+} (0.63) – Fe^{3+} (0.03) – Hg^{2+} (0.97), Fe^{3+} (0.02) – Cu^{2+} (0.24) – Ni^{2+} (0.63) – Hg^{2+} (0.97), Pb^{2+} (0.0) – Cu^{2+} (0.31) – Ni^{2+} (0.67) – Hg^{2+} (0.97).
S_{13}	Ni^{2+} (0.71) – Cu^{2+} (0.32) – Hg^{2+} (0.97), Fe^{3+} (0.03) - Cu^{2+} (0.33) – Hg^{2+} (0.97), Fe^{2+} (0.03) – Ni^{2+} (0.68) – Hg^{2+} (0.96), Fe^{2+} (0.02) – Cu^{2+} (0.32) – Ni^{2+} (0.72) – Hg^{2+} (0.97), Zn^{2+} (0.15) – Cd^{2+} (0.65) – Hg^{2+} (0.98), Pb^{2+} (0.0) – Cu^{2+} (0.31) – Ni^{2+} (0.71) – Hg^{2+} (0.97).
S_{14}	Fe^{3+}(0.05) – Cu^{2+} (0.25) – Ni^{2+} (0.65), Fe^{3+} (0.02) – Cu^{2+} (0.35) – Ni^{2+} (0.65) – Hg^{2+} (0.97), Zn^{2+} (0.14) – Cd^{2+} (0.67) – Hg^{2+} (0.96), Pb^{2+} (0.0) – Cu^{2+} (0.32) – Ni^{2+} (0.71) – Hg^{2+} (0.97).
S_{15}	Fe^{3+} (0.02) – Cu^{2+} (0.27) – Ni^{2+} (0.67), Fe^{3+} (0.02) – Cu^{2+} (0.25) – Hg^{2+} (0.95), Fe^{3+} (0.02) –Cu^{2+} (0.26) – Ni^{2+} (0.71) – Hg^{2+} (0.97), Zn^{2+} (0.12) – Cd^{2+} (0.60) – Hg^{2+} (0.91), Fe^{3+} (0.0) – Ni^{2+} (0.66)–Hg^{2+} (0.95), Pb^{2+} (0.0) – Cu^{2+} (0.27) – Ni^{2+} (0.61) – Hg^{2+} (0.95).
S_{16}	Zn^{2+} (0.11) – Cd^{2+} (0.58) – Hg^{2+} (0.98), Fe^{3+} (0.04) – Ni^{2+} (0.60) – Hg^{2+} (0.94), Pb^{2+} (0.0) - Ni^{2+} (0.65) - Hg^{2+} (0.97).

Table – 3.6

Physico-Chemical properties of different soil samples:

S.no	Place of collection	Physical parameter			Chemical parameter						
		Texture	pH	Electrical conductivity (ohm^{-1})	Cation exchange capacity	Carbon (%)	Organic matter (%)	K	Na	Ca	N	P
S_2	AMU fort Dhurrah, Aligarh	Sandy loam	8.30	0.874	3.6	0.56	0.97	30	15	1	0.29	28
(S_3,S_4)	i) Sewage water irrigated soil (15,20 cm)	Sandy loam	8.20	0.871	4	0.37	0.65	2	13	3	0.28	30
(S_5,S_6)	ii) Tubewell irrigated soil (15,20 cm)	Sandy loam	7.72	0.970	3.4	0.42	0.72	3	13	4	0.26	28
S_8	Tappal soil	Clay	8.20	0.874	3.7	0.89	1.54	8	47	20	0.36	40
S_7	Jattari soil	Loan	7.89	0.867	4	0.42	0.72	5	34	2	0.28	30
S_1	Botany deptt. soil	Sandy loam	7.70	0.869	6.9	0.63	1.87	39	16	1	0.26	29

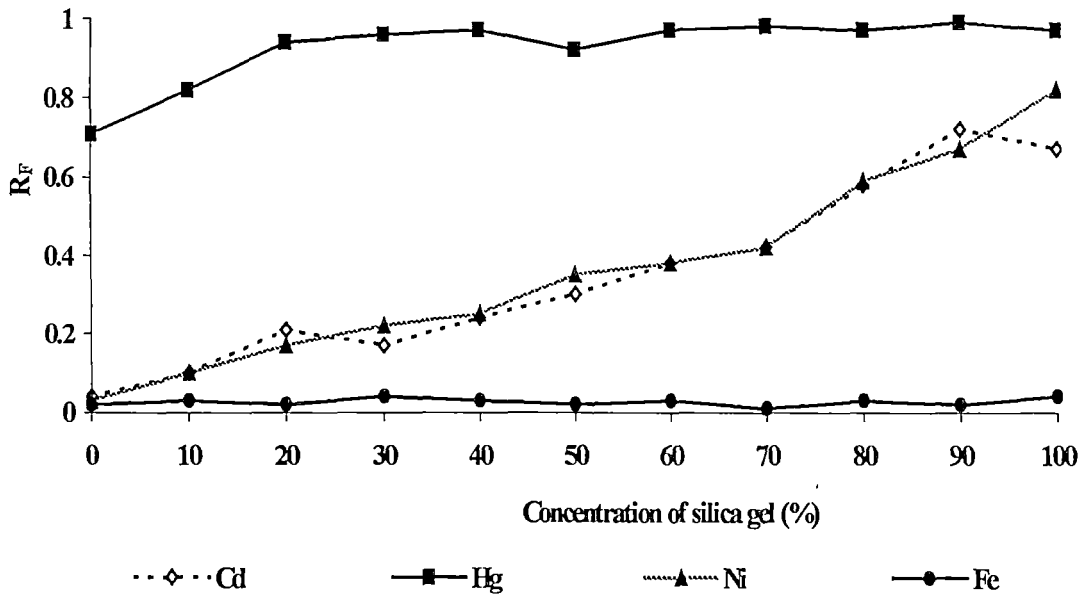

Fig.3.1: Effect on the mobility of certain metal ions by the amount of silica gel present in the mixed stationary phase containing soil.

CHAPTER-IV

Soil thin-layer chromatography of heavy metal cations with surfactant modified mobile phase: separation of coexisting zinc(II), *cadmium* (II) *and mercury* (II).

4.1 INTRODUCTION

The separation of heavy metal ions has attracted considerable attention in recent years, because of their environmental importance in aqueous media. Among separation techniques, thin-layer chromatography (TLC) is considered to be more application – oriented because of its versatility. The separation of ions on thin-layers is mainly influenced by ion exchange properties of the adsorbent and the coordinative properties of the solvent. In general practice, the composition of mobile phase is altered to achieve a desired separation on a particular adsorbent. According to the literature on inorganic TLC (1-5), mobile phases currently in use include (i) inorganic solvents (ii) organic solvents (iii) mixed aqueous – organic solvents (iv) complex-forming solvents.

Surfactant-containing aqueous mobile phases have received considerable attention in liquid chromatographic separation of organic and inorganic substances since the first report by Armstrong and Fendler (6) who exploited the favourable features of micellar systems in chromatography. The use of surfactant as one of the mobile phase components either below its critical micelle concentration (CMC) in ion pair chromatography (IPC) or above the CMC in micellar liquid chromatography (MLC) has been the focus of numerous studies (7-13).

A new concept of qualitative analysis by soil TLC was developed by Helling and Turner in 1968 (14). Their method was utilized by several workers to examine the mobility pattern of pesticides (15-18) and heavy metals (19-24) through various types of soil beds. The mobility or leachability of such chemicals through the bed has tremendous influence on the life process of plants. Metal pollutants from electroplating operations can find their environmental pathways through soil and water into plant and animal tissues. Metals tend to get adsorbed easily on surfaces of soil and sediments.

In view, of the above facts, it was considered worthwhile to examine the mobility of heavy metal cations through static planar bed of pure soil

using surfactant-mediated mobile phase systems. Hg and Cd are among the most toxic elements known to man. All the chemical compounds of Hg are toxic to humans (25). The highly labile behaviour of Cd is responsible for its accumulation in the human diet. Zn is an essential trace element for humans and animals, it acts as a catalytic or structural component in numerous enzymes involved in energy metabolism, but it causes adverse effect on the crops and human diets if present in high concentrations. The results of this study may be of immense utility to understand the role of surfactant as a component of aqueous mobile phase systems on the leachability of heavy metal cations through pure soil layers in order to restrict its bio availability in the soil-plant-animal pathway.

4.2 EXPERIMENTAL

Chemicals and Reagents:

Sodium dodecyl sulphate, SDS (B.D.H. India), urea (S.D, fine chemicals India) and inorganic salts such as sodium phosphate, ammonium chloride, sodium chloride, ammonium nitrate, potassium iodide (CDH, India) were of Analytical Reagent grade and used as such without further purification.

Metal Cations Studied:

As mentioned in chapter III.

Test Solutions:

As mentioned in chapter III.

Detection:

Similar chromogenic reagents were used for the detection of metal ions on soil TLC plates as given in chapter III.

Stationary Phase:

Pure soil was collected from Aligarh Muslim University, Aligarh (India) Farm. After the removal of grass and organic matters from the surface, Soil sample was obtained from the depth of 0 to 15 cms. The physico-chemical properties determined as per standard procedures are given in **Table 4.1**

Mobile phases:

Symbol		Composition
M_1	(a)	0.2 M NH_2CONH_2
	(b)	1.0 M NH_2CONH_2
	(a)	0.2 M NaH_2PO_4
M_2	(b)	1.0 M NaH_2PO_4
	(c)	2.0 M NaH_2PO_4
	(a)	0.2 M NH_4Cl
M_3	(b)	1.0 M NH_4Cl
	(c)	2.0 M NH_4Cl
	(a)	0.2 M NH_4NO_3
M_4	(b)	1.0 M NH_4NO_3
	(c)	2.0 M NH_4NO_3
	(a)	0.2 M $(NH_4)_2SO_4$
M_5	(b)	1.0 M $(NH_4)_2SO_4$
	(c)	2.0 M $(NH_4)_2SO_4$
	(a)	0.2 M NaCl
M_6	(b)	1.0 M NaCl
	(c)	2.0 M NaCl
	(a)	0.2 M KI
M_7	(b)	1.0 M KI
	(c)	2.0 M KI
	(a)	0.2 M $CaCl_2$
M_8	(b)	1.0 M $CaCl_2$
	(c)	2.0 M $CaCl_2$
	(a)	1.0 M NH_4Cl + 1.0 M KI (1:9)
M_9	(b)	1.0 M NH_4Cl + 1.0 M KI (1:1)
	(c)	1.0 M NH_4Cl + 1.0 M KI (9:1)
	(a)	1.0 M NH_4Cl + 1.0 M NaCl (1:9)
M_{10}	(b)	1.0 M NH_4Cl + 1.0 M NaCl (1:1)
	(c)	1.0 M NH_4Cl + 1.0 M NaCl (9:1)

M_{11}	(a)	1.0 M NH_4Cl + 1.0 M $CaCl_2$ (1:9)
	(b)	1.0 M NH_4Cl + 1.0 M $CaCl_2$ (1:1)
	(c)	1.0 M NH_4Cl + 1.0M $CaCl_2$ (9:1)
M_{12}	(a)	1.0 M NaH_2PO_4 + 1.0 M KI (1:9)
	(b)	1.0 M NaH_2PO_4 + 1.0 M KI (1:1)
	(c)	1.0 M NaH_2PO_4 + 1.0 M KI (9:1)
M_{13}	(a)	1.0 M NaH_2PO_4 + 1.0 M NaCl (1:9)
	(b)	1.0 M NaH_2PO_4 + 1.0 M NaCl (1:1)
	(c)	1.0 M NaH_2PO_4 + 1.0 M NaCl (9:1)
M_{14}	(a)	1.0 M NaH_2PO_4 + 1.0 M $CaCl_2$ (1:9)
	(b)	1.0 M NaH_2PO_4 + 1.0 M $CaCl_2$ (1:1)
	(c)	1.0 M NaH_2PO_4 + 1.0 M $CaCl_2$ (9:1)
M_{15}	(a)	0.1 M SDS + 1.0 M $CaCl_2$ (1:9)
	(b)	0.1 M SDS + 1.0 M $CaCl_2$ (1:1)
M_{16}	(a)	0.01 M SDS + 1.0 M $CaCl_2$ (1:9)
	(b)	0.01 M SDS + 1.0 M $CaCl_2$ (1:1)
	(c)	0.01 M SDS + 1.0 M $CaCl_2$ (9:1)
M_{17}	(a)	0.001 M SDS + 1.0 M $CaCl_2$ (1:9)
	(b)	0.001 M SDS + 1.0 M $CaCl_2$ (1:1)
	(c)	0.001 M SDS + 1.0 M $CaCl_2$ (9:1)
M_{18}		0.1 M SDS + 1.0 M KI (1:9)
M_{19}	(a)	0.01 M SDS + 1.0 M KI (1:9)
	(b)	0.01 M SDS + 1.0 M KI (1:1)
	(c)	0.01 M SDS + 1.0 M KI (9:1)
M_{20}	(a)	0.001 M SDS + 1.0 M KI (1:9)
	(b)	0.001 M SDS + 1.0 M KI (1:1)
	(c)	0.001 M SDS + 1.0 M KI (9:1)
M_{21}	(a)	0.1 M SDS + 1.0 M NaCl (1:9)
	(b)	0.1 M SDS + 1.0 M NaCl (1:1)
	(c)	0.1 M SDS + 1.0 M NaCl (9:1)
M_{22}	(a)	0.01 M SDS + 1.0 M NaCl (1:9)
	(b)	0.01 M SDS + 1.0M NaCl (1:1)
	(c)	0.01 M SDS + 1.0 M NaCl (9:1)

M_{23}	(a)	0.001 M SDS + 1.0 M NaCl (1:9)
	(b)	0.001 M SDS + 1.0 M NaCl (1:1)
	(c)	0.001 M SDS + 1.0 M NaCl (9:1)
M_{24}	(a)	0.1 M SDS + 2.0 M NaCl (1:9)
	(b)	0.1 M SDS + 2.0 M NaCl (1:1)
	(c)	0.1 M SDS + 2.0 M NaCl (9:1)
M_{25}	(a)	0.01 M SDS + 2.0 M NaCl (1:9)
	(b)	0.01 M SDS + 2.0 M NaCl (1:1)
	(c)	0.01 M SDS + 2.0M NaCl (9:1)
M_{26}	(a)	0.001 M SDS + 2.0 M NaCl (1:9)
	(b)	0.001 M SDS + 2.0 M NaCl (1:1)
	(c)	0.001 M SDS + 2.0 M NaCl (9:1)
M_{27}		0.001 M CTAB + 2.0 M NaCl (1:9)
M_{28}		0.001 M Brij-35 + 2.0 M NaCl (1:9)
M_{29}		0.001 M TX-100 + 2.0 M NaCl (1:9)

Salt solutions were prepared in double distilled water (conductivity = 2 x 10^6 ohm^{-1} cm^{-1}).

CHROMATOGRAPHY

Preparation of soil thin-layer plates

For preparation of soil TLC plates, with or without the removal of soil organic matter, similar procedure was followed as mentioned in chapter II.

Removal of organic matter

50 g of the air dried sample was taken into a 250 ml conical flask and 100 ml of 1 M ammonium acetate was added to it. The contents of the flask were shaken for 20 min and allowed to stand over night. The soil contents were then transferred into a buckner funnel for filtration. The soil was then leached with an additional 400 ml of ammonium acetate. The filtrate was evaporated to dryness. The residue containing organic matter was treated with a mixture of 2 ml of 50% hydrogen peroxide and 2 ml of 6N nitric acid and heated to dryness.

Procedure

As mentioned in chapter III.

4.3 RESULTS AND DISCUSSION

The results of the present study have been summarized in **Table 4.2-4.8**. With the exception of Cd^{2+} and Hg^{2+} none of the metal ions showed mobility and all remained near the point of application (R_F = 0.0-0.14) as highly compact spots in all the solvents systems used (M_1– M_{29}). The data listed in **Table 4.2** show the mobility trends of metal ions (Cd^{2+} and Hg^{2+}) on soil layers developed with different concentrations (0.2, 1.0 and 2.0M) of aqueous fertilizers and sodium, potassium and calcium salt solutions. When fertilizers (urea, sodium phosphate, ammonium chloride, ammonium nitrate and ammonium sulphate) at different concentration levels were used as mobile phase (M_1– M_5), both metal ions remained near the point of application. However, when soil TLC plates were developed with salt solutions (sodium chloride, potassium iodide and calcium chloride) Cd^{2+} and Hg^{2+} showed significant mobility. At low concentration level of sodium salt (0.2M), both metal ions remained near the point of application (R_F = 0.08 and 0.1 respectively) but at 1.0 M concentration level, Hg^{2+} moved near the middle of the chromatoplates (R_F = 0.66) whereas Cd^{2+} remained near the point of application (R_F = 0.2). On further increasing of sodium salt concentration (2.0 M), Cd^{2+} migrated near the middle of the chromatoplate (R_F = 0.45) and Hg^{2+} moved near the solvent front (R_F = 0.88). With 0.2 M aqueous solution of potassium salt used as mobile phase (M7a), Cd^{2+} exhibited moderate mobility (R_F= 0.42) whereas Hg^{2+} showed comparatively higher mobility (R_F = 0.74). At 1.0 and 2.0 M concentration levels of potassium salt solution both Cd^{2+} and Hg^{2+} showed approximately identical mobilities (R_F = 0.71, 0.79 and 0.69, 0.74 respectively). At 0.2 M concentration level of calcium salt solution, Cd^{2+} showed little mobility (R_F = 0.3) whereas at 1.0 and 2.0 M concentration, it showed an enhanced mobility (R_F = 0.72 and 0.75 respectively). At all concentration levels of calcium salt solutions (0.2 – 2.0 M), Hg^{2+} exhibited high but somewhat constant mobility (R_F = 0.87 – 0.93). Thus, it can be concluded that an increase in the R_F value of the Cd^{2+} is associated with the increase in concentration from 0.2 to 2.0 M of sodium, potassium or calcium salt in the mobile phase whereas, Hg^{2+} exhibited somewhat constant but higher R_F values. The interesting feature of the present study is that

all metal cations including Cd^{2+} and Hg^{2+} produced compact spots (i.e. $R_L - R_T <$ 0.30).

To examine the combined influence of fertilizers and inorganic salts in the mobile phase on the mobility of Cd^{2+} and Hg^{2+}, ammonium chloride or sodium phosphate (1.0 M) were mixed with 1.0 M solution of potassium, sodium or calcium salts in varying ratio (1:1, 1:9 and 9:1, v/v). The resultant mobile phase systems were used to determine the mobility of both Cd^{2+} and Hg^{2+} ions. The results obtained are summarized in **Table 4.3,** from where following conclusions may be drawn:

i. There occurs a significant decrease in the R_F value of Cd^{2+} with the increase in the amount of added fertilizers in the mobile phase irrespective of the type of inorganic salt present in the eluent.

ii. The R_F value of Hg^{2+} fluctuates between 0.69 and 0.97 with all the solvent systems (M_9 – M_{14}) used. The exceptions were M_{10}(c) and M_{11}(a) where Hg^{2+} showed R_F values of 0.26 and 0.12 respectively. Thus, it can be stated that presence of fertilizers in the mobile phase causes little effect on the movement (or R_F value) of Hg^{2+} whereas, it has retarding effect on the mobility of Cd^{+2}.

The mobility of metal ions was also examined using different concentrations of SDS (0.001 – 0.8 M). All the metal ions remained near the point of application (R_F = 0.03) irrespective of the fact whether SDS concentration is below (0.001 M) or above (0.8M) its critical micelle concentration (CMC). The CMC value of SDS in water is 0.008 M. Thus, pure aqueous SDS solution cannot be used as mobile phase for separation studies on soil layers as SDS fails to induce differential migration among the metal cations. Therefore, it was decided to use SDS with added salt solutions as mobile phase, to examine the mobility of metal ions on pure soil bed. For this purpose mobile phase systems (M15 – M20) were prepared by mixing aqueous solutions of calcium or potassium salts (1.0 M) and aqueous SDS solutions (0.001 – 0.1 M) in 1:1, 1:9 and 9:1 ratios. Similarly, mobile phase systems M21 – M26 were obtained by mixing aqueous sodium chloride (1.0 or 2.0 M) with aqueous SDS (0.001 – 0.1 M) in 1:1, 1:9 and 9:1

ratios. The results obtained with these mobile phase systems have been listed in **Tables 4.4 and 4.5.** From the available data following conclusions may be drawn.

i. Cd^{2+} gave mid R_F (R_F = 0.34 – 0.58) and Hg^{2+} showed higher mobility (R_F = 0.75 – 0.85) in all the solvent systems used with the exception of mobile phases M_{16}(c) and M_{17}(c) where Cd^{2+} remained somewhat near the point of application and Hg^{2+} moved marginally giving R_F value of 0.35 and 0.25 respectively.

ii. When mobile phases M_{18} – M_{20} were used, Cd^{2+} moved from the point of application to reach around the middle of the chromatoplate (R_F = 0.47 – 0.78) in all the solvent systems used with the exception of mobile phase M_{19}(c) where it remained near the point of application (R_F = 0.19). However, Hg^{2+} exhibited comparatively higher mobility (R_F – 0.67 – 0.82) in all the solvent systems used.

iii. When mobile phase systems M_{21} – M_{23} were used, Cd^{2+} spot appeared slightly above the point of application giving the maximum value of R_F as 0.34. However, Hg^{2+} showed considerably higher mobility and moved near or well above the middle of the chromatoplate (R_F = 0.42 – 0.82) with the exception of mobile phase M_{21}(c) where it showed very little mobility (R_F = 0.3). On further increasing of sodium salt concentration to 2.0 M (M_{24} - M_{26}), Cd^{2+} exhibited moderate mobility or gave elongated spots whereas, Hg^{2+} showed comparatively higher mobility. When SDS at the concentration 0.001M was mixed with 2.0M sodium salt solution in the ratio 1:9 [M_{26}(a)] best results from separation point of view, clarity and sharpness of spot detection were observed with this mobile phase system which facilitates an analytically important separation of Zn^{2+} (R_F = 0.05) from Cd^{2+} (R_F = 0.51) and Hg^{2+} (R_F = 0.85). Hence, this particular solvent system [M_{26}(a)] was used for detailed TLC studies.

The R_F values of Cd^{2+}, Hg^{2+} and Zn^{2+} obtained in 0.001 M aqueous SDS solution with added KI, NaCl or $CaCl_2$ salt solution have been tabulated in **Table 4.6.** These results clearly indicate that the separation of coexisting Cd^{2+}, Hg^{2+} and

Zn^{2+} ions is always possible with mobile phases M_{17}(a), M_{23}(a) and M_{26}(a). However, better resolved spots were realised with M_{26}(a).

To observe the effect of cationic and non-ionic surfactants on the mobility of Zn^{2+}, Cd^{2+} and Hg^{2+}, the anionic SDS surfactant was replaced by cationic surfactant (CTAB) and non-ionic (TX-100 or Brij-35) surfactant in the chosen mobile phase i.e. M_{26}(a) and the resulted mobile phases (M_{27} – M_{29}) were used to determine the mobility of Zn^{2+}, Cd^{2+} and Hg^{2+} on soil layer. The results obtained with M_{27}- M_{29} have been summarized in **Table 4.7**. These results demonstrate that Zn^{2+} always remained near the point of application irrespective of nature of the surfactant. Cd^{2+} appeared as compact spot with M_{27} but it gave tailed spots with M_{28} and M_{29}. Though compact spots were realized for Hg^{2+} with M_{27} and M_{28} but it gave badly tailed spot with M_{29}. Thus, these mobile phase systems were not found suitable for the separation of Zn^{2+} from Cd^{2+} and Hg^{2+} as evident from the results of Table 4.7.

To examine the effect of different inorganic additives and acids on the mobility of Zn^{2+}, Cd^{2+} and Hg^{2+}, sodium molybdate, potassium thiocyanate, oxalic acid, tartaric acid and citric acid (1.0 M) were added in 1:9 and 9:1 ratio by volume into the chosen mobile phase [M_{26}(a)]. From the data listed in **Table 4.8** following trends are noticeable:

(i) With sodium molybdate and potassium thiocyanate additives the mobility of Zn^{2+} remained unaffected and the mobility of Cd^{2+} is lowered at all concentrations of the additives. In case of Hg^{2+} its mobility is only decreased from 0.85 to 0.07 when the concentration of sodium molybdate in the mobile phase was 90%. This lowering in R_F value of Hg^{2+} may be attributed to the complexation of Hg^{2+} with molybdate at higher concentration of the latter.

(ii) The mobility of Zn^{2+} is enhanced at both concentration levels of acids.

(iii) At higher concentration of carboxylic acids (i.e. 90%) an enhanced mobility of Cd^{2+} was observed.

Thus, the presence of additives in the mobile phase influences the separation of Zn^{2+} from Cd^{2+} or Hg^{2+}.

Soil organic matter causes an increase in the pH of the soil, with the increase in pH the cation exchange property of soil increases and so does its preference for polyvalent cations, it leads to the formation of stable complexes. Hence, the presence of soil organic matter effects the mobility of metal ions which are developed on pure soil layers. To illustrate this affect, TLC plates coated with organic matter free soil were used for chromatography of metal cations using selected solvent system [M_{26}(a)] as mobile phase. It was observed that most of the metal ions showed significant mobility (R_F = 0.55 – 0.87) with the exception of Fe^{3+}, Cu^{2+} and Ag^{+} which remained near the point of application. Co^{2+} could not be detected clearly. The results of ΔR_F values (ΔR_F = R_F on organic matter free soil layer – R_F on organic matter containing soil layer) are presented in **Figure 4.1.** The positive ΔR_F values are indicative of high mobilities of metal ions on soil layers free from organic matter. With organic matter containing soil layers used as stationary phases all the metal ions with the exception of Hg^{2+} and Cd^{2+} remained near the point of application (R_F = 0.03). Hg^{2+} and Cd^{2+} gave R_F values of 0.85 and 0.54 respectively. Thus, organic matter free soil layer used as stationary phase contributes significantly to the mobility of metal ions.

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Table 4.1

Physico – chemical properties of the soil sample used as stationary phase.

Place of collection	Physical parameter			Chemical parameter								
	Texture	pH	Electrical conductivity (ohm^{-1} cm^{-1})	Cation exchange capacity meq(100g)	Organic carbon (%)	Organic matter (%)	K (mg/l)	Na (mg/l)	N (%)	P (%)	Ca (%)	Mg (%)
A.M.U. farm	Sandy loam	8.11	1.0227	14.8	0.852	1.4814	20	30	2.01	1.96	0.039	0.356

Table 4.2

Mobility (R_F value of Cd^{2+} and Hg^{2+} developed with 0.2, 1.0 and 2.0 M concentration of different fertilizers and salt solutions

Mobile phase		Cd^{2+}	Hg^{2+} *
	(a)	0.07	0.05
M_1**	(b)	0.06	0.05
	(a)	0.05	0.07
M_2	(b)	0.10	0.06
	(c)	0.10	0.05
	(a)	0.09	0.00
M_3	(b)	0.14	0.08
	(c)	0.10	0.07
	(a)	0.07	0.04
M_4	(b)	0.12	0.03
	(c)	0.10	0.05
	(a)	0.07	0.08
M_5	(b)	0.06	0.09
	(c)	0.12	0.13
	(a)	0.08	0.10
M_6	(b)	0.20	0.66
	(c)	0.45	0.88
	(a)	0.42	0.74
M_7	(b)	0.71	0.69
	(c)	0.79	0.74
	(a)	0.30	0.87
M_8	(b)	0.72	0.93
	(c)	0.75	0.87

* *The difference in R_L (R_F of leading front) and the R_T (R_F of traling front) values in case of Cd^{2+} and Hg^{2+} was always less than 0.30 & hence the spots were considered compact.*

** *Development of plates in 2.0 M concentration level of urea is not feasible due to prolong development time*

Table 4.3

R_F values of metal ions (Cd^{2+} and Hg^{2+}) developed with 1.0 M ammonium chloride or sodium phosphate mixed in various ratios with 1.0 M potassium iodide, sodium chloride and calcium chloride

Mobile phase		Cd^{2+}	Hg^{2+}
	(a)	0.70	0.72
M_9	(b)	0.69	0.69
	(c)	0.11	0.71
	(a)	0.38	0.79
M_{10}	(b)	0.13	0.71
	(c)	0.23	0.26
	(a)	0.22	0.12
M_{11}	(b)	0.14	0.75
	(c)	0.15	0.85
	(a)	0.83	0.91
M_{12}	(b)	0.79	0.86
	(c)	0.16	0.89
	(a)	0.38	0.80
M_{13}	(b)	0.11	0.71
	(c)	0.18	0.77
	(a)	0.38	0.97
M_{14}	(b)	0.23	0.80
	(c)	0.21	0.79

Table 4.4

R_F values of Cd^{2+} and Hg^{2+} developed with 1.0 M calcium chloride or potassium iodide mixed with different concentrations of SDS in varying ratios

Mobile phase		Cd^{2+}	Hg^{2+}
M_{15}*	(a)	0.52	0.76
	(b)	0.36	0.85
	(a)	0.58	0.83
M_{16}	(b)	0.34	0.77
	(c)	0.21	0.35
	(a)	0.47	0.79
M_{17}	(b)	0.53	0.75
	(c)	0.20	0.25
M_{18}**		0.67	0.79
	(a)	0.70	0.74
M_{19}	(b)	0.78	0.77
	(c)	0.19	0.82
	(a)	0.67	0.73
M_{20}	(b)	0.66	0.72
	(c)	0.47	0.67

* *Solvent system 0.1 M SDS + 1.0 M $CaCl_2$ (9:1) could not be used for the development of TLC plates due to formation of precipitation*

** *Solvent systems 0.1 M SDS + 1.0 M KI (1:1) and 0.1 M SDS + 1.0 M KI (9:1) could not be used for the development of TLC plates due to the formation of precipitation*

Table 4.5

Mobility trend (R_F values) of Cd^{2+} and Hg^{2+} developed in solvent systems comprising of 1.0 or 2.0 M sodium chloride mixed with different concentrations of SDS in varying ratios

Mobile phase		Cd^{2+}	Hg^{2+}
M_{21}	(a)	0.25	0.75
	(b)	0.20	0.66
	(c)	0.18	0.30
M_{22}	(a)	0.20	0.78
	(b)	0.18	0.68
	(c)	0.10	0.44
M_{23}	(a)	0.34	0.82
	(b)	0.22	0.65
	(c)	0.10	0.42
M_{24}	(a)	0.48	0.71
	(b)	0.22T	0.75
	(c)	0.32T	0.52
M_{25}	(a)	0.49	0.75
	(b)	0.28T	0.72
	(c)	0.25T	0.50
M_{26}	(a)	0.54	0.85
	(b)	0.43	0.53
	(c)	032	0.50

T = Tailed Spot ($R_L - R_T > 0.30$)

Table 4.6

R_F values of Cd^{2+}, Hg^{+2} and Zn^{2+} developed with 0.001 M SDS containing $CaCl_2$ or KI (1.0 M) and NaCl (1.0 or 2.0 M) in 1:9 ratio

Metal ions	R_F Value			
	$M_{17}(a)$ (0.001 M SDS + 1.0 M $CaCl_2$, 1:9)	$M_{20}(a)$ (0.001M SDS + 1.0 M KI, 1:9)	$M_{23}(a)$ (0.001 M SDS + 1.0 M NaCl, 1:9)	$M_{26}(a)$ (0.001 M SDS + 2.0 M NaCl, 1:9)
Cd^{2+}	0.47	0.67	0.34	0.54
Hg^{2+}	0.79	0.73	0.82	0.85
Zn^{2+}	0.06	0.07	0.14	0.02

Table 4.7

Effect of replacing cationic and non-ionic surfactants in the solvent systems M_{26}(a) on the mobility (R_F values) of Zn^{2+}, Cd^{2+} and Hg^{2+}

Mobile phase	R_F values		
	Zn^{2+}	Cd^{2+}	Hg^{2+}
M_{27}	0.04	0.53	0.80
M_{28}	0.12	0.46T	0.87
M_{29}	0.05	0.52T	0.52T

Table 3

Separation possibilities of Zn^{2+}, Cd^{2+} and Hg^{2+} in the presence of inorganic and carboxylic acids as impurities in the selected mobile phase $M_{26}(a)$

Mobile Phase	R_F values			Remarks
	Zn^{2+}	Cd^{2+}	Hg^{2+}	
$M_{26}(a)$	0.02	0.54	0.85	Good ternary separation of Zn^{2+}, Cd^{2+} and Hg^{2+}
Sodium molybdate + $M_{26}(a)$				
1:9	0.03	0.14	0.85	Binary separation of Hg^{2+} from Zn^{2+} and Cd^{2+}
9:1	0.05	0.04	0.07	No possible separation
Potassium thiocyanate + $M_{26}(a)$				
1:9	0.07	0.52	0.75	Binary separation of Hg^{2+} from
9:1	0.07	0.19	0.74	Zn^{2+} or Cd^{2+}
Oxalic acid + $M_{26}(a)$ *				
1:9	0.21	0.47	0.85	Poor separation possibility of Zn^{2+}
9:1	0.17	0.55	0.79	from Cd^{2+} and of Cd^{2+} from Hg^{2+}
Citric acid + $M_{26}(a)$				
1:9	0.53	0.50	0.70	No separation of Zn^{2+} from Cd^{2+}
9:1	0.70	0.72	0.67	No separation of Zn^{2+} from Cd^{2+}
Tartaric acid + $M_{26}(a)$				
1:9	0.40	0.47	0.89	No separation of Zn^{2+} from Cd^{2+}
9:1	0.51	0.70	0.78	No separation of Cd^{2+} from Hg^{2+}

* ΔR_F (R_F Cd^{2+} - R_F Zn^{2+}) = 0.26

ΔR_F (R_F Hg^{2+} - R_F Cd^{2+}) = 0.24,

For good separation $\Delta R_F > 0.30$ has been considered.

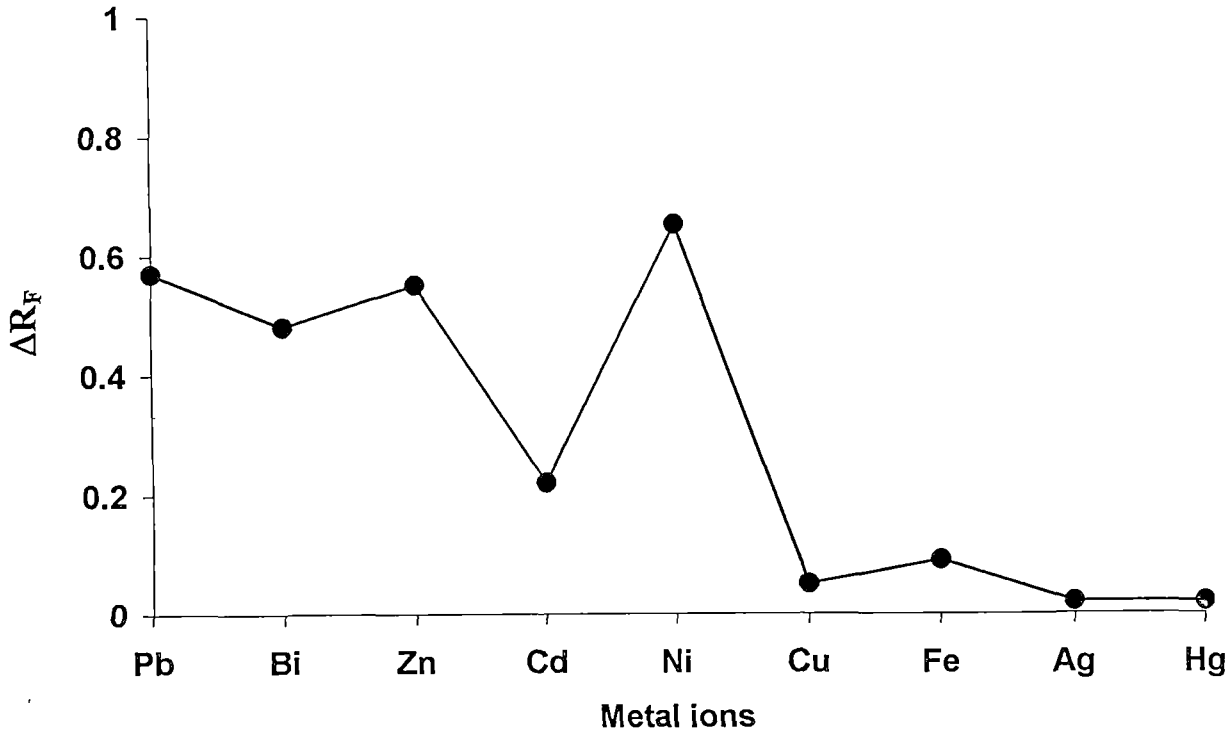

Figure 4.1 Plot of ΔR_F vs Metal ions

$\Delta R_F = R_F$ on organic matter free soil layer - R_F on organic matter containing soil layer

CHAPTER-V

Separation studies of transition metal ions with cationic micellar eluents by normal phase thin-layer chromatography.

5.1 INTRODUCTION

Over the years, thin layer chromatography (TLC) has become a preferred analytical technique for the separation of closely related inorganic species using aqueous (acidic, neutral and alkaline solvents) and mixed organic or aqueous-organic solvent systems as chromatographic eluents. It has been successfully utilized in the analysis of wastewater for total heavy metal contents (1), characterization of hazardous wastes (2), estimation of toxic metals in industrial sewage (3) and the recovery of thiocyanate from photogenic wastewater (4). Addition of aqueous micellar solutions pioneered by Armstrong and Terrill (5) as mobile phase systems has further accentuated the importance of TLC where simultaneous separation of ionic or non-ionic species in a variety of matrices is required. Micellar liquid chromatography (MLC) where the concentration of a surfactant in mobile phase is kept above the critical micelle concentration (CMC) of the surfactant has gained immense popularity and wider applicability due to operational simplicity, cost effectiveness, relatively non-toxicity and enhanced separation efficiency (6-10).

We recently reported (11-14) the chromatographic separation of inorganic species on silica gel and alumina layer, with surfactant-mediated mobile phase systems. It was found that better separation possibilities for cationic and anionic species were on silica gel and alumina layers respectively. As regards to the nature of surfactant in the mobile phase, uncommon separation selectivity for cations was realized with cationic surfactant eluents. Conversely, eluents containing anionic surfactant were found most suitable for selective separation of anionic species. In continuation of our earlier studies, we have used cetyl trimethylammonium bromide (CTAB), a cationic surfactant as eluent to achieve rapid separation of coexisting Hg^{2+}, Cd^{2+} and Zn^{2+} ions from aqueous solutions on silica layer. Simultaneous separation of Hg^{2+}, Cd^{2+} and Zn^{2+} bears environmental, biological and metallurgical importance because zinc is frequently associated with cadmium and one metal contains small

quantities of the others with deleterious effects on performance. It is therefore not surprising that several analytical techniques including ion-exchange chromatography (15), reversed – phase paper chromatography (16), solvent extraction (17) and TLC (11, 18) have been used for the separation of Cd^{2+} from Zn^{2+}. However, none of these studies involve the use of surfactant-mediated mobile phase systems. The present work was therefore undertaken to utilize the chromatographic performance of cationic surfactant in the separation of heavy metal cations.

5.2 EXPERIMENTAL

Chemicals and Reagents:

Silica gel 'G', sodium dodecyl sulfate (SDS), dimethylglyoxime, methanol, ethanol, propanol and butanol were of Qualigens, India; alumina, N-cetyl-N,N,N-trimethyl ammonium bromide (CTAB), potassium ferrocyanide, dithizone and 1,10-phenanthroline were of CDH, India. All other chemicals were also of Analytical Reagent grade.

Metal Ions Studied:

As mentioned in Chapter III.

Test Solutions:

As described in Chapter III.

Detection:

As described in Chapter III.

Stationary Phase:

Silica gel 'G'

Mobile Phases: The following solvent systems were used as mobile phase

No.	Symbol	Composition
(a)	M_1	Water
(b)	Aqueous surfactant solutions	
	M_2	1% aqueous CTAB
	M_3[a]	3% aqueous CTAB
	M_4	5% aqueous CTAB
	M_5	7% aqueous CTAB
	M_6	1% aqueous SDS
	M_7	3% aqueous SDS
	M_8	5% aqueous SDS
	M_9	7% aqueous SDS
(c)	Buffered micellar solutions	
	M_{10}	3% CTAB in buffer solution of pH 2.3
	M_{11}	3% CTAB in buffer solution of pH 4.0
	M_{12}	3% CTAB in buffer solution of pH 5.7
	M_{13}	3% CTAB in buffer solution of pH 9.0
	M_{14}	3% CTAB in buffer solution of pH 11.9
(d)	Aqueous micellar solutions with added NaCl	
	M_{15}	3% aqueous CTAB containing 1g NaCl per 100 mL
	M_{16}	3% aqueous CTAB containing 5g NaCl per 100 mL
	M_{17}	3% aqueous CTAB containing 10g NaCl per 100 mL
	M_{18}	3% aqueous CTAB containing 15g NaCl per 100 mL
(e)	Aqueous micellar solution with added urea	
	M_{19}	3% aqueous CTAB containing 1g urea per 100 mL
	M_{20}	3% aqueous CTAB containing 5g urea per 100 mL
	M_{21}	3% aqueous CTAB containing 10g urea per 100 mL
	M_{22}	3% aqueous CTAB containing 15g urea per 100 mL
(f)	Aqueous micellar solution with added alcohol	
	M_{23}	3% aqueous CTAB + methanol (20+80)*
	M_{24}	3% aqueous CTAB + methanol (50+50)
	M_{25}	3% aqueous CTAB + methanol (80+20)
	M_{26}	3% aqueous CTAB + ethanol (20+80)
	M_{27}	3% aqueous CTAB + ethanol (50+50)
	M_{28}	3% aqueous CTAB + ethanol (80+20)
	M_{29}[b]	3% aqueous CTAB + propanol (20+80)
	M_{30}	3% aqueous CTAB + propanol (50+50)
	M_{31}[c]	3% aqueous CTAB + propanol (80+20)
	M_{32}	3% aqueous CTAB + butanol (90+10)
	M_{33}	3% aqueous CTAB + butanol (95+5)

* Mobile phase proportions for multi-solvent systems are in parts by volume

a) CMC = 0.46%, b) CMC = 0.34%, c) CMC = 0.36%

of the amount of metal ion until no spot was detected. The minimum amount detectable on the TLC plates was taken as the limit of detection.

Recovery of Cadmium by Spot-Area Method:

To determine recovery of cadmium by spot – area measurement method, 0.01 mL from a series of standard solutions of Cd^{2+} (0.5 – 2.5%) were spotted on silica layers. The plates were developed with M31. After detection, the spots were copied onto tracing paper from the chromatoplates and then the area of each spot was calculated. The recovery of cadmium was studied by analyzing various samples spiked with Cd^{2+}. For this purpose, experiments were performed by spotting 0.01 mL of sample solution containing 100 μg cadmium. The recovery of Cd^{2+} was 82 ± 2%.

5.3 RESULTS AND DISCUSSION

The results of this study have been summarized in **Tables 5.1- 5.5** and **Figures 5.1-5.2.** The mobility of ten metal cations was examined on silica gel layer using aqueous solutions of SDS and CTAB. In order to optimize the experimental conditions, effect of various factors, such as (a) concentration of the surfactants, (b) acidity and basicity of the medium, (c) the presence of urea and NaCl in the surfactant – containing mobile phase and (d)) nature of the added alkanols in the micellar mobile phase on the mobility of cations was examined.

(a) Effect of Concentration of Surfactants

To understand the effect of concentration of cationic (CTAB) or anionic (SDS) surfactant on the mobility of metal cations, chromatography was performed on silica layers using different concentrations of surfactant - mediated mobile phase systems (M_2–M_9) as developers. The R_F values of metal cations obtained in pure water (i.e. zero surfactant concentration, M_1) and in aqueous solutions of CTAB and SDS at different concentration levels (M_2–M_9) are listed in **Table 5.1**. From the data listed in Table 5.1, following conclusions may be drawn:

CHROMATOGRAPHY

(a) ***Preparation of TLC Plates***: As described in Chapter II.

(b) ***Preparation of Spiked, Industrial Wastewater and River Water***: A 20 mL of industrial wastewater (pH, 2.97) collected from lock industries, Aligarh, India or river water (pH, 7.48) obtained from Ganga river at Naraura, India or sea water (pH, 8.47) obtained from Anjana beach, Goa, India was spiked with 100 μg each of Zn, Cd and Hg salts. About 20 mL of 0.5% thioacetamide solution was added into spiked sample. The resultant precipitate of Zn, Cd and Hg sulfides was washed with distilled water, centrifuged and dissolved in minimum possible volume of concentrated HCl. The acid was completely evaporated and the residue was dissolved in 5 mL of distilled water. An aliquot (10 μL) of each sample was applied on TLC plate and chromatography was performed as done for the standard samples.

(c) ***Preparation of Synthetic Ores***: Cinnabar (HgS) , zinc blende (ZnS) and greenoekite (CdS) were synthetically prepared by spiking 50 mL of distilled water (pH 5.6) with Zn, Cd and Hg salt solutions following the same procedure as described above in (b).

Procedure: As mentioned in Chapter III.

Separation:

For the separation, equal amounts of metal ions to be separated were mixed and 10μL of the resultant mixture was spotted on the activated TLC plate which was then dried in air. The plates were developed to a distance of 10 cm, the spots were detected and the separated metal cations were identified on the basis of R_F values.

Limits of Detection:

The limits of detection of metal cations were determined by spotting different amounts of metal ion on the TLC plates, developing the plates and detecting the spot. The method was repeated with successive lowering

of the amount of metal ion until no spot was detected. The minimum amount detectable on the TLC plates was taken as the limit of detection.

Recovery of Cadmium by Spot-Area Method:

To determine recovery of cadmium by spot – area measurement method, 0.01 mL from a series of standard solutions of Cd^{2+} (0.5 – 2.5%) were spotted on silica layers. The plates were developed with M31. After detection, the spots were copied onto tracing paper from the chromatoplates and then the area of each spot was calculated. The recovery of cadmium was studied by analyzing various samples spiked with Cd^{2+}. For this purpose, experiments were performed by spotting 0.01 mL of sample solution containing 100 μg cadmium. The recovery of Cd^{2+} was 82 ± 2%.

5.3 RESULTS AND DISCUSSION

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(a) Effect of Concentration of Surfactants

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(i) In pure water, all metal ions except Ni^{2+}, Co^{2+} and Cd^{2+} show little mobility and Ag^{+} was found to produce tailed spot. The higher mobility of Ni^{2+} (R_F = 0.90) and Co^{2+} (R_F = 0.85) facilitates their separations from all other metal ions studied whereas the mid R_F (R_F=0.40) of Cd^{2+} opens opportunities for its selective separation from multicomponent mixture of metal cations.

(ii) When aqueous surfactant (SDS or CTAB) solutions at different concentration levels (1, 3, 5 and 7%) were used as mobile phase, metal ions such as Fe^{3+}, Pb^{2+}, Bi^{3+}, Zn^{2+} and Ag^{+} stayed very close to the point of application. Conversely, Ni^{2+} and Co^{2+} show higher mobility ($R_F \approx 0.85$).

(iii) Metal ions such as Ni^{2+}, Cd^{2+}, Ag^{+} and Bi^{3+} show occasional tailing ($R_L - R_T > 0.3$) and their spots appear as elliptical extension in the direction of solvent flow.

(iv) Cu^{2+} shows higher mobility in CTAB (M_3–M_5) compared to its mobility in SDS (M_6–M_9).

(v) Cd^{2+} produces badly tailed spots at all concentration levels of surfactant irrespective of the nature of the surfactant (cationic or anionic).

(vi) Hg^{2+} shows higher mobility in the presence of cationic surfactant, but in case of SDS it produces tailed spot.

(vii) 3% aqueous CTAB solution (M_3) was judged to be favourable for further studies as more compact spots for cations were realized with this mobile phase system.

(j) Effect of Acidity and Basicity of the Medium

Thin layer chromatography of metal ions was performed using 3% CTAB solution prepared in borate buffers of different pH values (pH = 2.3, 4.0, 5.7, 9.0 and 11.9). The results obtained with the buffered surfactant solutions have been summarized in **Table 5.2**. The following conclusions may be drawn from these results:

(i) Change in pH of mobile phase system had little effect on the mobilities of Fe^{3+}, Hg^{2+}, Ag^{+}, Zn^{2+}, Bi^{3+} and Pb^{2+}. Except Bi^{3+} and Hg^{2+}, all these metal ions remained near the point of application (R_F = 0.10) irrespective of the fact whether mobile phase medium used was of acidic or basic in nature. Bi^{3+} produced tailed spots at all pH values and Hg^{2+} showed higher mobility (R_F = 0.88 – 0.96).

(ii) With strongly acidic mobile phases (M_{10} – M_{11}), Cu^{2+} showed relatively higher mobility (R_F, 0.32 at pH 2.3 and 0.22 at pH 4.0) while it remained near the point of application with weakly acidic (M_{12}) or alkaline (M_{13} – M_{14}) mobile phase systems.

(iii) Cd^{2+} exhibited a peculiar behaviour as it gave double spots (R_F = 0.32, 0.69) with mobile phase M_{10} (pH = 2.3), tailed spot (R_F = 0.85) with M11 (pH = 4.0) and exceptionally well formed compact spots with relatively higher R_F value with M_{12} (R_F = 0.79), M_{13} (R_F = 0.82) and M_{14} (R_F = 0.89).

(iv) Compact spots of Ni^{2+} and Co^{2+} were visualized in highly acidic (M_{10}) or basic medium (M_{13} – M_{14}). However, when the nature of the mobile phase was less acidic (pH = 4.0 or 5.7) both these metal ions gave elongated spots (R_F = 0.85).

(i) Effect of Non-electrolyte and Electrolyte Additives

The effect of addition of urea (organic non electrolyte) and NaCl (inorganic electrolyte) at different concentration levels in the eluent M_3 (3% aqueous CTAB) on the mobility of metal ions was examined. The results obtained with NaCl added mobile phases (M_{15}–M_{18}) and urea added mobile phase systems (M_{19}–M_{22}) are summarized in **Table 5.3**. From the available data, following trends are noticeable:

(i) The mobility of Fe^{3+} remained almost unaffected at all concentration levels of urea and NaCl. The mobility of Ag^{+} was found to increase with the increase in NaCl concentration. However, their mobilities in the presence of urea remained almost unchanged.

(ii) Co^{2+} exhibited slight increase in mobility with the increase of urea concentration in the mobile phase. However, the presence of NaCl caused no effect on the mobility of this metal ion ($R_F \pm 0.95$).

(iii) Ni^{2+} and Cd^{2+} produced tailed spots at all concentration levels of urea in contrast to the formation of highly compact spots with NaCl added mobile phases regardless of the NaCl concentration.

(j) Effect of Alkanols

To investigate the retention of metal cations on silica layer, hybrid - mobile phase systems comprising of micelle (3% CTAB) – water – alcohol (methanol, ethanol, propanol or butanol) were also used and better chromatographic performance in terms of promotion of differential migration of metal ions by these mobile phases over aqueous micellar mobile phase systems was observed. The values of CMC determined for M_3 (3% aqueous CTAB), M_{29} (3% aqueous CTAB + 80% propanol) and M_{31} (3% aqueous CTAB + 20% propanol) were 0.46%, 0.34% and 0.36% respectively. It shows that propanol decreases the CMC value of CTAB from 0.46% to 0.34%. Hence, CTAB is present in the form of micelles. The R_F values of metal cations were determined with micelle–water–alcohol mobile phases (M_{23}–M_{33}) consisting of methanol (20, 50 or 80%), ethanol (20, 50 or 80%), propanol (20, 50 or 80%) or butanol (5 or 10%) and 3% aqueous CTAB. In all cases, a general trend of decreasing R_F value with the increase in alcohol concentration was noticed. The representative's plots are given in **Figures 5.1(a) and (b).** The retention mechanism is connected with the adsorption of surfactant on the surface of the stationary phase. As long as the surface of the silica gel layer is saturated by the adsorbed surfactant i.e. there are no more free silanol groups on the layer for the interaction, metal ions move faster showing higher R_F value. The addition of alcohol to the mobile phase leads to the reduction in the adsorbed amount of surfactant on the stationary phase (19), which causes an increase in retention of solutes and a change in selectivity. Thus, in the presence of alcohol the silica layers are not

completely saturated by adsorbed surfactant and there are still free silanol groups, the interaction between them and the metal cations affects the retention pattern. In these cases, the nature and the polarity of alcohol play an important role in the separation. In general, the better separation possibilities were realized with mobile phase containing 20% propanol (M_{31}). Successful separation of Zn^{2+}, Cd^{2+} and Hg^{2+} can be achieved with any of the mobile phase containing alcohols. However, mobile phase M_{31} is most favourable. We successfully separated coexisting Zn^{2+}, Cd^{2+} and Hg^{2+} on silica layer using M_{31} as mobile phase. The R_F values of the resolved spots were in the order Hg^{2+} (0.95), Cd^{2+} (0.47) and Zn^{2+} (0.07). Thus, moderately polar alcohol (e.g propanol) provides superior separation of metal cations compared to more polar alcohol (e.g methanol).

It has been reported (20) that the microenvironment of micellar system is greatly influenced by the presence of added organic substance and inorganic electrolytes. To examine this aspect, inorganic (NaCl, NaBr, $NaNO_3$, HCOONa, $NaHCO_3$, NH_4Cl, NH_4Br and NH_4NO_3) and organic (oxalic, citric, tartaric acid, urea, L-arginine, L-valine, L-tryptophan, L-methionine, L-serine and L-glutamic acid) impurities were added in M_{31} to obtain mobile phase systems containing 1% of each foreign substance. The resultant mobile phase systems were used to examine the effect of impurities in the eluent on simultaneous separation of Zn^{2+}, Cd^{2+} and Hg^{2+}. The results presented in **Table 5.4** clearly demonstrate that mutual separation of Zn^{2+}, Cd^{2+} and Hg^{2+} could not be achieved with mobile phases containing 1% citric, oxalic or tartaric acid because of the co-migration of Zn^{2+} and Cd^{2+} with Hg^{2+}. Similarly, with other mobile phase systems the separation of Cd^{2+} from Hg^{2+} is hampered. Conversely, its separation from Zn^{2+} is improved because of the enhanced mobility of Cd^{2+}. This observation suggests that the presence of organic or inorganic impurities in water-micelle-alcohol hybrid mobile phase systems have serious adverse effects on chromatographic performance of micellar mobile phases in regards to the separation of metal cations.

The lowest possible detectable microgram amount alongwith dilution limit of metal cation (given in parenthesis) on silica gel layer were, Zn^{2+} (0.017, 1: 5.8×10^5), Cd^{2+} (0.50, 1: 2×10^4) and Hg^{2+} (0.05, 1 : 2×10^5).

Semiquantitative estimation of Cd^{2+}

An attempt has been made to determine the recovery of cadmium spiked into water using spot-area measurement method. A linear relationship obtained when the amount of sample spotted was plotted against area of the spot follows the empirical equation $\xi = km$, where ξ is the area of the spot, m is the amount of the solute and k is a constant. Representative plot for Cd^{2+} has been shown in **Figure 5.2**. The linearity is maintained upto 250 μg /spot. At higher concentration a positive deviation from linear law was observed. The accuracy and precision was around ± 12%.

Application:

The proposed method was applied for identification of zinc, cadmium and mercury in spiked industrial wastewater, river and sea water samples as well as in metal sulphide ores after separation on silica layer. The results listed in **Table 5.5** clearly demonstrate that Hg, Cd and Zn which are spiked in the various samples can be recovered and separated on silica TLC plates developed with M_{31}.

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Table 5.1

R_F value of metal cations obtained on silica layers developed with pure water (M_1) and aqueous solutions of CTAB (M_2-M_5) and SDS (M_6-M_9) at different concentration levels

Metal ions	R_F value								
	Water	CTAB				SDS			
	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9
Fe^{3+}	0.10	0.05	0.06	0.07	0.10	0.06	0.05	0.02	0.05
Cu^{2+}	0.10	0.07	0.27	0.32	0.37	0.05	0.07	0.07	0.10
Ni^{2+}	0.90	0.85T	0.85T	0.85T	0.85T	0.87	0.90	0.87T	0.85T
Co^{2+}	0.85	0.72	0.72	0.77	0.80	0.87	0.90	0.90	0.85T
Cd^{2+}	0.40	0.45T	0.63T	0.80T	0.85T	0.45T	0.80T	0.82T	0.82T
Zn^{2+}	0.05	0.06	0.15	0.20T	0.20	0.07	0.10	0.10	0.10
Ag^{+}	0.30T	0.00	0.11	0.15T	0.17T	0.07	0.10	0.07	0.02
Pb^{2+}	0.02	0.02	0.07	0.10	0.15T	0.02	0.05	0.00	0.02
Bi^{3+}	0.04	0.07	0.12	0.15T	0.16T	0.15T	0.15T	0.15T	0.10
Hg^{2+}	0.02	0.95	0.95	0.95	0.95	0.33T	0.65T	0.65T	0.64T

T = Tailed Spot ($R_L - R_T > 0.30$)

Table 5.2

Mobility of metal cations developed with buffered surfactant solutions of different pH values

Metal ions	R_F value				
	M_{10}	M_{11}	M_{12}	M_{13}	M_{14}
Fe^{3+}	0.07	0.10	0.11	0.07	0.10
Cu^{2+}	0.32	0.22	0.05	0.15	0.02
Ni^{2+}	0.18	0.85T	0.85T	0.86	0.82
Co^{2+}	0.81	0.85T	0.85T	0.86	0.75
Cd^{2+}	0.32,0.69	0.85T	0.79	0.82	0.89
Zn^{2+}	0.12	0.04	0.07	0.16	0.11
Ag^{+}	0.08	0.05	0.05	0.07	0.10
Pb^{2+}	0.05	0.05	0.05	0.05	0.09
Bi^{3+}	0.32T	0.40T	0.30T	0.20T	0.35T
Hg^{2+}	0.88	0.95	0.96	0.95	0.96

Table 5.3

Mobility of metal cations on 3% aqueous CTAB containing different concentrations of electrolyte and non-electrolyte additives

	R_F value							
	NaCl added mobile phase				Urea added mobile phase			
Metal ions	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{20}	M_{21}	M_{22}
Fe^{3+}	0.05	0.06	0.06	0.06	0.05	0.07	0.05	0.05
Cu^{2+}	0.22	0.27	0.20T	0.20T	0.22	0.22	0.22	0.27
Ni^{2+}	0.92	0.92	0.90	0.92	0.85T	0.84T	0.85T	0.85T
Co^{2+}	0.92	0.92	0.90	0.95	0.70	0.77	0.80	0.82
Cd^{2+}	0.90	0.92	0.92	0.94	0.55T	0.57T	0.60T	0.65T
Zn^{2+}	0.10	0.07	0.20T	0.20T	0.07	0.12	0.10	0.12
Ag^{+}	0.00	0.06	0.25T	0.37	0.10	0.10	0.06	0.11
Pb^{2+}	0.07	0.22T	0.87	0.65T	0.05	0.04	0.02	0.02
Bi^{3+}	0.06	0.09	0.12	0.15T	0.06	0.05	0.10	0.12
Hg^{2+}	0.92	0.92	0.90	0.90	0.92	0.92	0.95	0.90

T = Tailed Spot ($R_L - R_T > 0.30$)

Table 5.4

Separation of coexisting Zn^{2+}, Cd^{2+} and Hg^{2+} in the presence of inorganic and organic impurities.

Stationary phase: silica gel Mobile phase: M_{31}

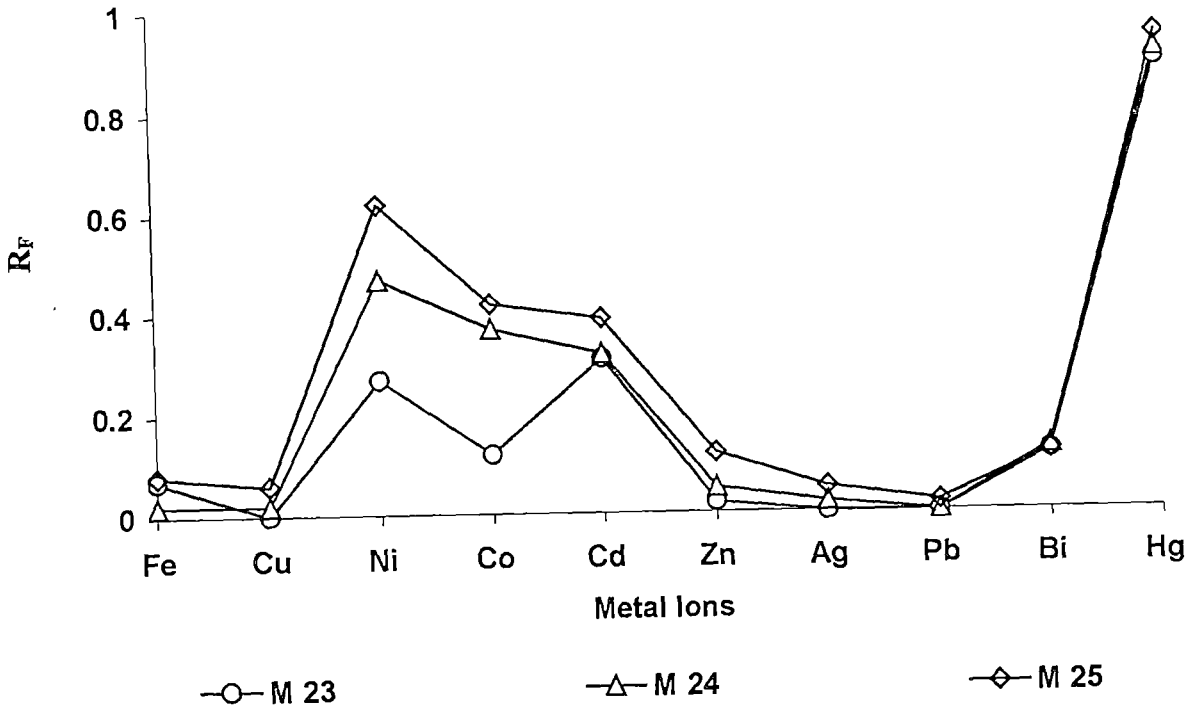
Additives (1%)	Separation (R_F)		
	Zn^{2+}	Cd^{2+}	Hg^{2+}
NaCl	0.07	0.90	0.95
NaBr	0.08	0.80	0.95
$NaNO_3$	0.09	0.60	0.95
HCOONa	0.10	0.71	0.98
$NaHCO_3$	0.02	0.20T	0.97
NH_4Cl	0.08	0.67	0.95
NH_4Br	0.07	0.70	0.95
NH_4NO_3	0.07	0.70	0.95
Oxalic acid	0.69	0.92	0.95
Citric acid	0.71	0.80	0.95
Tartaric acid	0.72	0.95	0.97
Urea	0.10	0.40	0.95
L-arginine	0.07	0.50	0.95
L-valine	0.11	0.56	0.94
L-tryptophan	0.07	0.62	0.95
L-methionine	0.11	0.57	0.95
L-serine	0.09	0.45	0.94
L-glutamic acid	0.50	0.67	0.92
Without additives	0.07	0.47	0.95

Table 5.5

Recovery and separation of coexisting Zn^{2+}, Cd^{2-} and Hg^{2+} ions from spiked water and synthetically prepared metal ores and heavy metal sludge samples

Stationary phase: silica gel Mobile phase : M31

Spiked/synthetic samples	R_F value		
	Zn^{2+}	Cd^{2+}	Hg^{2+}
River water	0.05	0.46	0.95
Sea water	0.03	0.46	0.95
Sulphides	0.14	0.56	0.96
Distilled water	0.07	0.47	0.95



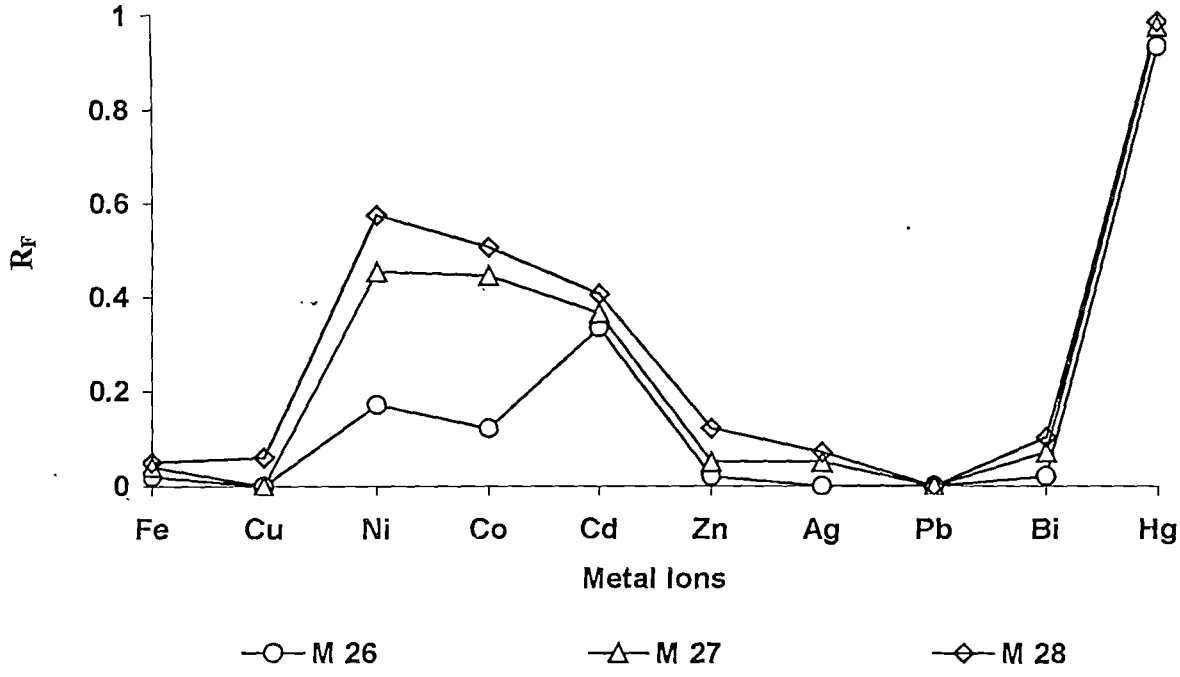
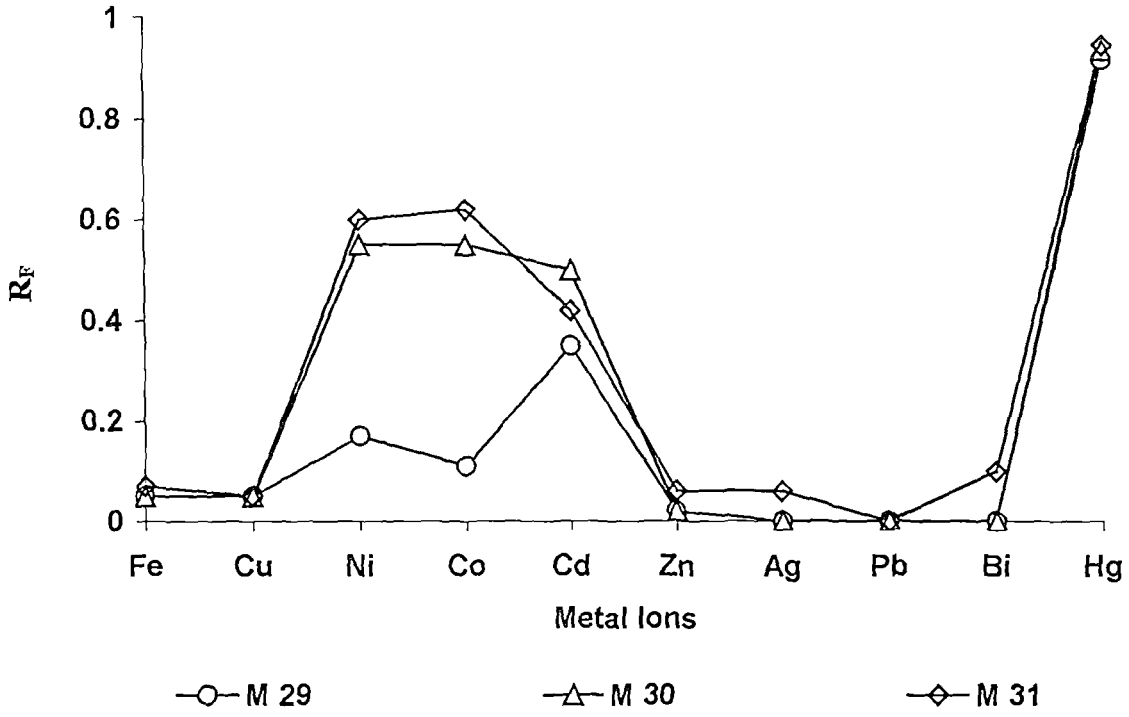


Figure 5.1(a) Mobility of heavy metal cations on silica gel layers developed with 3% aqueous CTAB containing different amounts of alcohols.



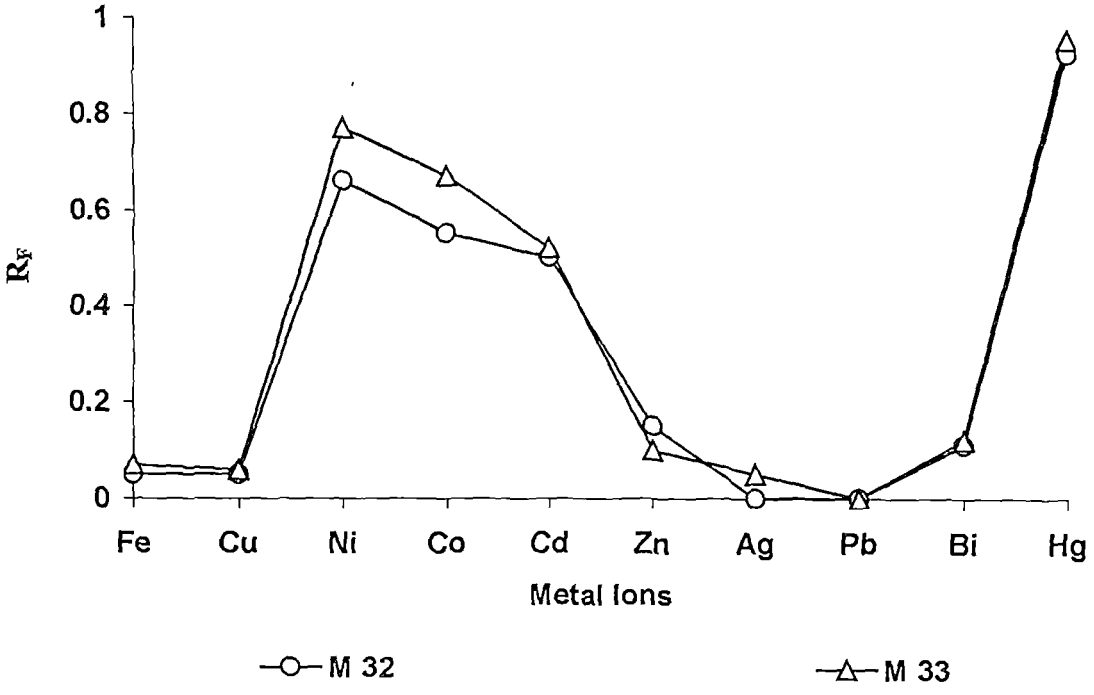


Figure 5.1(b) Mobility of heavy metal cations on silica gel layers developed with 3% aqueous CTAB containing different amounts of alcohols.

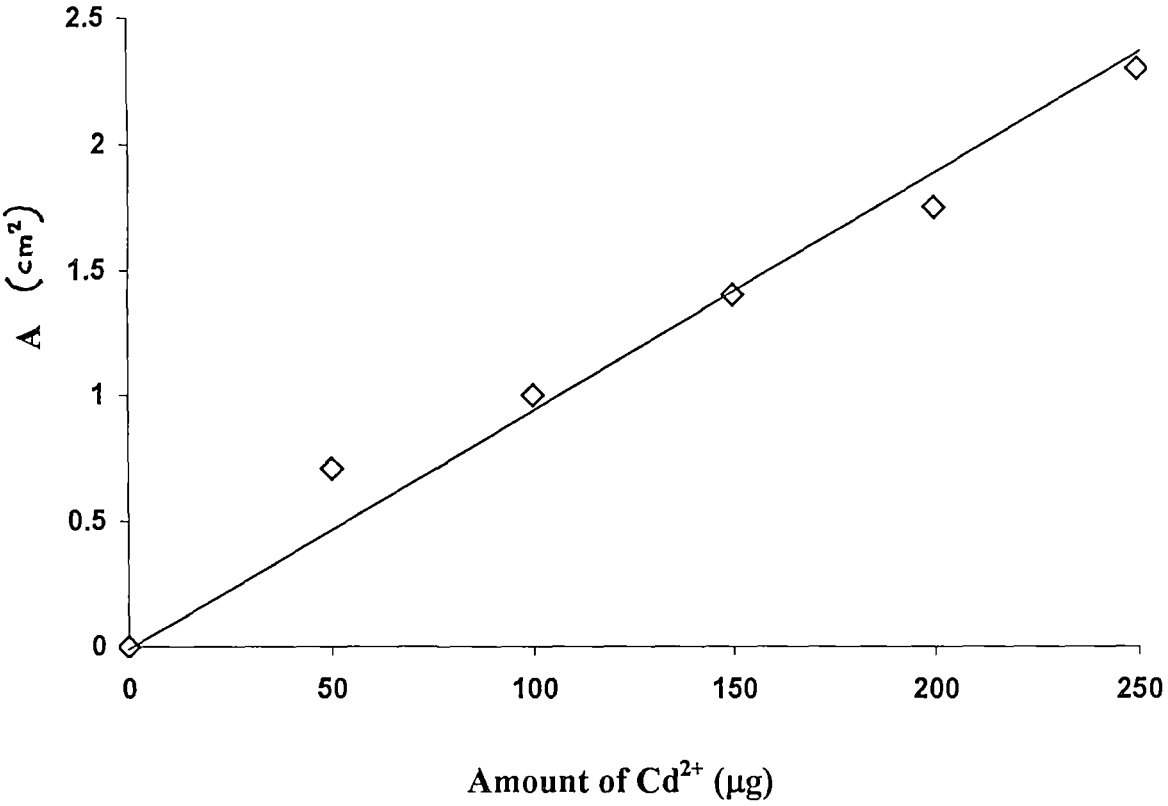


Figure 5. 2 Calibration curve for semiquantitative determination of Cd^{2+}

Stationary phase: silica gel

Mobile phase: M_{31}

CHAPTER-VI

Use of surfactant as extractant for separation of Cr^{6+} from Cr^{3+} and associated metal cations.

6.1 INTRODUCTION

Heavy metals have received considerable attention of chemists in recent past because of their physiological and environmental importance (1–2). According to our present state of knowledge metals such as Pb, Cd, Hg, Ni, Cu, Zn, As and Cr are toxic and harmful to human health. These metals are capable to form stable complexes with bio–ligands containing oxygen, nitrogen, or sulphur atoms (3) and control several redox processes in living organism. The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in aquatic life. Industrial waste constitutes the major source of various kinds of metal pollution in aqueous systems. Heavy metals cannot be degraded or detoxified biologically. The wastes containing heavy metals are extremely pernicious due to the fact of being environmentally persistent. The major sources of chromium to the aquatic environment are electroplating and metal finishing industrial effluents, sewage and wastewater treatment plant discharge, and chromates from cooling water. Chromium exists in several oxidation states (eg. di–, tri–, penta–, and hexa–) but only Cr^{3+} and Cr^{6+} are biologically important.

Hexavalent chromium is hazardous to biological activities. Ferrochrome and chrome–steel production industrial wastes are important sources of Cr^{6+} pollution. The major source of Cr^{3+} pollution is leather tanning waste effluent. Water containing Cr^{6+} above 0.05mg L^{-1} is dangerously toxic both to the mammals and aquatic lives (4), while Cr^{3+} above 5mg L^{-1} is toxic only to fish (5). Cr^{6+} has been estimated in ores and alloys by hydrated Sn^{4+} and Zr^{4+} oxides (6) after separation from other constituents. The various analytical techniques available for the detection, determination and separation of chromium include normal phase and reversed–phase thin–layer chromatography (7–9), ion–chromatography (10–11), extraction chromatography (12), ion–exchange chromatography (13–14), reversed–phase high performance liquid chromatography (15–16), micellar electrokinetic chromatography (17), precipitation floatation (18),

solid–phase extraction (19), titrimetry (20–21), capillary electrophoresis (22–23), spectrophotometry (24–25), atomic absorption spectroscopy (26–27), atomic emission spectroscopy (28), neutron activation analysis (29–30), flame atomic absorption spectroscopy (31–32), graphite furnace atomic absorption spectroscopy (33–34) and hyphenated techniques such as ion– exchange chromatography– flame atomic absorption spectroscopy (35), ion-chromatography-thermal lens spectrometry (36–37), gas chromatography–neutron activation analysis (38), inductively coupled plasma mass spectroscopy–atomic emission spectroscopy (39–42), ion exchange chromatography–flame atomic absorption spectroscopy (43), solid–phase extraction–flame atomic emission spectroscopy (44), liquid chromatography–inductively coupled plasma mass spectroscopy (45), high performance liquid chromatography–inductively coupled plasma mass spectroscopy (46), and ion–chromatography– inductively coupled plasma mass spectroscopy (47).

Of the various separation procedures, thin–layer chromatography (TLC) is probably the most versatile as it can be used for the selective separation of metal cations on micro as well as macro scale. The use of high–performance (HP) TLC plates has further enhanced the efficiency of this technique.

Micellar liquid chromatography (MLC) involving the use of surfactant ions above their critical micelle concentration (CMC) as mobile phase has been the focus of numerous studies (48–52) for controlling the retention of a variety of solutes since its inception in 1977 by Armstrong and co–workers (53). Taking into consideration the advantageous features such as inexpensiveness, non–toxicity non–flammability, unique separation capabilities, enhanced detection sensitivity and fast analysis of micellar mobile phases, it was decided to utilize the analytical potential of cationic N–cetyl–N,N,N–trimethyl ammonium bromide (CTAB) micellar solution as eluent in combination with precoated silica HPTLC plates as stationary

phase for achieving analytically important separation of Cr^{3+} from Cr^{6+}. The separation of different valency states of chromium is industrially important as Cr^{3+} is converted into Cr^{6+} in alkaline peroxide media. Conversely, Cr^{6+} is reduced to Cr^{3+} in the presence of I^-ion with the liberation of I_2. Traditionally, the separation efficiency of micellar systems has been enhanced by adding small quantities of organic additives e.g. 1–propanol or 1–pentanol (54,55), but the present investigation deviates from earlier studies as we have achieved improved separation with aqueous CTAB micellar solution in the absence of organic modifiers.

6.2 EXPERIMENTAL

Chemicals and Reagents:

Silica gel 60 F_{254} 'HPTLC' plates (Merck, Darmstadt, Germany); sodium dodecyl sulfate (SDS) (BDH, India); N–cetyl–N,N,N–trimethyl ammonium bromide (CTAB) (CDH, India); Triton x–100 and Brij–35 (Loba–Chemie, India); urea (S.D. Fine Chemicals Ltd., India) were used. All other reagents used were also of Analytical Reagent grade and used as such without further purification.

Test Solution:

Standard aqueous solutions (1.0%) of the chloride, nitrate or sulphate salts of Ni^{2+}, Co^{2+}, Hg^{2+}, Cd^{2+}, Pb^{2+}, Tl^{+}, Bi^{3+}, Al^{3+}, Ag^{+}, VO^{2+}, UO_2^{2+}, Mn^{2+}, Fe^{3+}, Cu^{2+}, Ti^{4+} and Mo^{6+} were used as test solutions. All the solutions were prepared in demineralized water with a specific conductivity ($k=2x10^{-6}$ ohm^{-1} at 25°C). To prevent the hydrolysis small quantities of corresponding acids were added to solutions of the nitrates of lead, silver and bismuth and the chloride of mercury.

Preparation of Test Solutions of Cr^{3+} and Cr^{6+}

Standard solution (1.0%) of Cr^{3+} and Cr^{6+} were prepared by dissolving 1g of chromium (III) chloride hexahydrate, $CrCl_3.6H_2O$, and potassium dichromate ($K_2Cr_2O_7$) respectively in 100 ml of distilled water.

Detection:

The reagents used for detection of the cations were, aqueous potassium ferrocyanide (1%) for Fe^{3+}, Cu^{2+}, UO_2^{2+}, VO^{2+} and Ti^{4+}; alkaline solution of dimethylglyoxime (0.2%) for Ni^{2+} and Co^{2+}; aqueous aluminon (1%) for Al^{3+}; 1% methanolic solution of alizarin red 's' for Cr^{3+}; 1% yellow ammonium sulfide dissolved in ammonia for Pb^{2+}, Hg^{2+}, Cd^{2+}, Tl^{+}, Bi^{3+}. 2.0 M NaOH+ 30% H_2O_2 mixed in 1:1 ratio by volume for Mn^{2+}; 1% ferric chloride solution for Mo^{6+}. Cr^{6+} was self detected but alternatively it was detected with saturated alcohlic solution of Ag NO_3.

Stationary Phase: Silica gel 60 F_{254} 'HPTLC' plates.

Mobile Phase: The following solvent systems were used as mobile phase.

Symbol	Composition
I. Single surfactant containing mobile phase	
M_1	0.2M SDS
M_2	0.008M SDS
M_3	0.0032M SDS
M_4	0.0225M CTAB
M_5	0.0009M CTAB
M_6	0.000036 M CTAB
M_7	0.07M TX-100
M_8	0.0028 M TX–100
M_9	0.000112 M TX–100
M_{10}	0.0025 M Brij–35
M_{11}	0.0001 M Brij–35
M_{12}	0.000004 M Brij–35
II Mixed surfactant mobile phase Systems	
M_{13}	CTAB (0.0225M)+Brij–35 (0.0025M),1:1

Symbol	Composition
M_{14}	SDS (0.2M)+ Brij–35 (0.0025M), 1:1
M_{15}	CTAB (0.025M)+TX–100(0.07M), 1:1
M_{16}	SDS (0.2M)+TX–100(0.07M),1:1
III Surfactant systems with added urea	
$M_{17(a)}$	CTAB (0.0225M)+urea (1.0M), 1:9
$M_{17(b)}$	CTAB(0.0225M)+urea (1.0M), 9:1
$M_{18(a)}$	SDS(0.2M)+urea (1.0M), 1:9
$M_{18(b)}$	SDS (0.2M)+urea(1.0M), 9:1
$M_{19(a)}$	TX–100(0.07M)+urea(1.0M),1:9
$M_{19(b)}$	TX–100 (0.07M)+urea(1.0M),9:1
$M_{20(a)}$	Brij–35(0.0025M)+urea(1.0M), 1:9
$M_{20(b)}$	Brij–35 (0.0025M)+urea (1.0M), 9:1
$M_{21(a)}$	CTAB (0.0225M)+urea(2.0M), 1:9
$M_{21(b)}$	CTAB(0.0225M)+urea(2.0M), 3:7
$M_{21(c)}$	CTAB(0.0225M)+urea(2.0M),5:5
$M_{21(d)}$	CTAB(0.0225M)+urea(2.0M),7:3
$M_{21(e)}$	CTAB(0.0225M)+urea(2.0M),9:1
$M_{22(a)}$	SDS(0.2M)+urea (2.0M), 1:9
$M_{22(b)}$	SDS(0.2M)+urea(2.0M),3:7
$M_{22(c)}$	SDS(0.2M)+urea(2.0M),5:5
$M_{22(d)}$	SDS(0.2M)+urea(2.0M),7:3
$M_{22(e)}$	SDS(0.2M)+urea(2.0M), 9:1
$M_{23(a)}$	TX–100(0.07M)+urea(2.0M),1:9
$M_{23(b)}$	TX–100(0.07M)+urea(2.0M),9:1
$M_{24(a)}$	Brij–35 (0.0025M)+urea(2.0M),1:9
$M_{24(b)}$	Brij–35 (0.0025M)+ urea (2.0M), 9:1

Mobile phase proportions for multi–solvent systems are in parts by volume.

CHROMATOGRAPHY:

Test solutions (5μL) were applied on high performance thin layer plates with the help of microsyringe. The plates were developed in the chosen solvent system by the ascending technique. The solvent ascent was fixed to 5cm in all cases for the determination of R_F value of individual metal cations. After development, the plates were withdrawn from glass jars and dried at room temperature.

Suitable detection reagents were then sprayed on HPTLC plates for locating the position of cations. The R_L (R_F of leading front) and R_T (R_F of trailing front) values for each spot were determined and the R_F value was calculated.

$$R_F = \frac{R_L + R_T}{2}$$

Separation:

The test solution (0.01 mL) containing two or more metal ions to be separated were spotted on HPTLC plates and the chromatography was performed using various mobile phases. The resolved spots for these metal cations were observed on HPTLC plates after spraying chromogenic reagents and the R_{AM} values of the separated metal ions were determined. The new term R_{AM} used in this paper refers to the difference between R_T of the upper spot and R_L of the lower spot.

R_{AM} = (R_T of the upper spot–R_L of the lower spot).

Effect of pH:

To study the effect of pH of the test samples on the separation of Cr^{6+} from Cr^{3+}, analyte samples containing Cr^{6+} and Cr^{3+} in 1:1 ratio were brought to different pH values (pH 1.0–9.9) by the addition of required volumes of NaOH or dil. HCl and 0.01 mL of the resultant sample solutions was applied on the HPTLC plates. The plates were developed with M_4 and the R_F values of Cr^{6+} and Cr^{3+} were calculated after detection.

Limit of Detection:

The limit of detection of Cr^{3+} and Cr^{6+} were determined by spotting different amounts of metal cations on the HPTLC plates, developing the plates and detecting the spots using appropriate reagents. The method was repeated with successive lowering of amount of metal cations until no spots were detected. The minimum amount of metal cations detectable on the HPTLC plates was taken as the limit of detection.

Semiquantitative Determination by Spot–Area Measurement:

For semiquatitative determination by spot–area measurement method, 0.01 ml volumes from a series of standard solutions (0.5–2.0%) of Cr^{3+} and Cr^{6+} were spotted on HPTLC plates. The plates were developed with M_4 (0.0225M CTAB). After detection, the spots were copied onto tracing paper from the chromatoplates and then the area of each spot was calculated.

Chromatography of Spiked Wastewater Samples:

(a) *Preparation of spiked sample of industrial wastewater-* As the real sample of wastewater contains Cr^{6+}, hence it was spiked only with 1% aqueous solution of Cr^{3+}. For this purpose, equal volumes of wastewater and 1% Cr^{3+} solution were mixed together in 1:1 volume ratio. From the resultant spiked sample, an aliquot (0.01mL) was spotted on silica HPTLC plates and the plates were developed with M_4 and R_F values of the resolved spots of Cr^{6+} and Cr^{3+} were determined.

(b) *Preparation of synthetic alloys–* Cobalt alloy (Cr^{6+} 30%, Co 62% and Mn 8%) and nichrome (Cr^{6+} 20%, Fe 20% and Ni 60%) were synthetically prepared by mixing 1% salt solutions of Cr^{6+}, Co and Mn in 3.0:6.2:0.8 ratio and 1% salt solutions of Cr^{6+}, Fe and Ni in 2:2:6 ratio respectively.

Aliquots (5 mL) of above mentioned synthetic cobalt alloy and nichrome samples were spiked with 1.0 ml of 1% Cr^{3+} salt solution and the

resultant spiked sample (0.01 mL) were applied on two different HPTLC plates and chromatography was performed.

6.3 RESULTS AND DISCUSSION

The results of the present study have been summarized in **Tables 6.1–6.8** and **Figs. 6.1–6.2.** The mobilities of metal cations were assessed with aqueous solutions of cationic, anionic and non–ionic surfactants above, near and below their critical micelle concentration (CMC) and the results so obtained have been listed in **Table 6.1.** The CMC values of surfactants in water, given in parenthesis are SDS (0.008M), CTAB (0.0009M), Brij–35 (0.0001M) and TX–100(0.0028M) respectively. Aqueous solutions with surfactant concentrations either 25 times higher or 25 times lower than their CMC value were chosen for investigating mobility of metal cations, as higher surfactant concentration levels (50 or 100 times) poses difficulties like prolong development time and solubility limitations. With aqueous micellar SDS mobile phase (M_1), none of the metal ions showed significant mobility (R_F=0.0–0.12) with the exception of Cr^{6+}, M_0^{6+} Tl^+ and Hg^{2+}. Tl^+ showed mobility (R_F=0.71) and Cr^{6+} exhibits tailed spot. With mobile phases M_1–M_{12}, M_0^{6+} showed high and constant mobilities irrespective of the fact whether the concentrations of surfactants were above, near or below their CMC. Hg^{2+} showed R_F value of 0.66 with aqueous micellar CTAB solution (M_4). Among all the surfactants used, aqueous cationic CTAB micellar solution (M_4) was found to be best in terms of clarity of detection and compactness of spots of the metal cations. Good separation of Cr^{6+} from Cr^{3+} was visualized in this particular mobile phase (M_4).

Mixed surfactant systems have received considerable interest both in theoretical and applied studies because of their technological and commercial applications. Mixtures of surfactants often exhibit synergism in their physico-chemical properties, thus allowing peculiar separation possibilities and spectrophotometric determination of inorganics (56, 57). This has prompted us to examine the effect of mixed surfactant systems on

the mobility of metal cations, for this purpose mobile phases M_{13}–M_{16} were obtained by mixing micellar solution of non–ionic, [Brij–35 (0.002M) or TX–100 (0.07M)] with cationic [CTAB (0.0225M)] or anionic [SDS (0.2M)] surfactants, in 1:1 ratio by volume. The migration rates of Cr^{6+}, M_0^{6+}, Tl^+ and Hg^{2+} obtained using the resultant mixed micellar solvent systems are encapsulated in **Table–6.2** in terms of R_F value. From these results following mobility trends were observed.

(i) Cr^{6+} exhibited tailed spot in SDS containing mixed micellar systems (M_{14} and M_{16}) , this behaviour is similar to that observed with aqueous micellar SDS mobile phase (M_1) system. However, the R_F value (i.e. mobility) of Tl^+ was decreased from 0.71 (M_1) to 0.43 (M_{14}) and 0.29 (M_{16}) indicating significant effect of mixed micellar systems containing SDS and a non–ionic surfactant. New separations such as Tl^+–Cr^{3+}, Al^{3+}–M_0^{6+}, Tl^+–Fe^{3+}, Cu^{2+}–MO^{6+}, Tl^+–Cd^{2+} and Ag^+– Mo^{6+} could be achieved with mixed micellar systems, such separations were not possible when aqueous solutions of single surfactants were used as eluent.

(ii) Hg^{2+} which showed significant mobility with aqueous micellar CTAB (M_4) to give R_F value of 0.66 was strongly retained and remained near the point of application (R_F=0.00–0.06) in CTAB containing mixed micellar solvent system (M_{13} and M_{14}) facilitating better separation of Hg^{2+} from Cr^{6+} or Mo^{6+} specially with M_{13}.

These observations may be attributed to the fact that when ionic and non–ionic surfactants are mixed together to form mixed micellar molecules, non–ionic surfactant penetrates between the molecules of ionic surfactants, hence causing lowering of R_F values (58).

Urea is known to form molecular associates in aqueous solutions and hence it alters the water structure (59). Therefore, the presence of urea in an aqueous surfactant solution may have effect on the nature and extent of aggregation of surfactant molecules (60) and hence may alter their

properties. To observe this effect mobile phases M_{17}–M_{24} were prepared by mixing urea (1.0 or 2.0M) with aqueous solutions of surfactants (CTAB, SDS, Brij–35 or TX–100) in different volume ratios. The data has been listed in **Tables 6.3 and 6.4**. From the results obtained it is apparent that with mobile phases M_{17}-M_{24} all metal ions (Ni^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Bi^{3+}, Ag^{+}, Cr^{3+}, VO^{2+}, UO_2^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}, Cu^{2+} and Ti^{4+}) remained near the point of application ($R_F \approx$ 0.00–0.10) with the exception of Mo^{6+}, Cr^{6+}, Tl^{+} and Hg^{2+}. Mo^{6+} exhibited constant mobility with R_F value ranging from 0.92–0.96 (M_{17}–M_{24}). However, in case of Cr^{6+}, Tl^{+} and Hg^{2+} addition of urea (1.0M or 2.0M) in the mobile phase brings about significant changes in the mobility (R_F value) which has been discussed below:

(i) With mobile phases M_{17}–M_{20} Cr^{6+} showed high mobility (R_F=0.91–0.96) Tl^{+} remained near the point of application with the exception of mobile phase M_{18}(a) and (b) where, it showed mobility with R_F value 0.32 and 0.57 respectively. Hg^{2+} showed mobility only with M_{17}(a) and (b) (R_F=0.35 and 0.65 respectively) where as in mobile phases M_{18}–M_{20} Hg^{2+} remained near the point of application (R_F 0.04–0.07).

(ii) Addition of urea (1.0M) in higher ratio with aqueous micellar CTAB or SDS solution (M_{17}a or M_{18}a) causes a decrease in the R_F values of Hg^{2+} (R_F=0.35) or Tl^{+} (R_F=0.32) respectively. This descent in the mobility of Hg^{2+} or Tl^{+} was more significant when urea at concentration level 2.0M was mixed with aqueous CTAB or SDS micellar solutions. Therefore, mobile phases M_{21}b–M_{21}d or M_{22}b–M_{22}d were prepared to illustrate a trend in decrease of mobilities of Hg^{2+} or Tl^{+} as shown in **Figs 6.1a and b**.

(iii) Elongated spot of Cr^{6+} is visualized as well formed compact spot with the addition of urea (1.0 or 2.0M) in aqueous micellar SDS solution. [M_{18}, M_{22}(a) and M_{22}(e)]

(iv) Compactness of Cr^{6+} spot is converted into elongated shape when urea (2.0M) was mixed in greater proportion with aqueous micellar CTAB solution ($M_{21}a$).

Lowering of R_F values of Tl^+ or Hg^{2+} as mentioned in (ii) may predominantly be due to the water structure breaking effect of urea (61). The contrast in the behaviour of Cr^{6+} as mentioned above in (iii) and (iv) may be attributed to the partial charge neutralization (62) of surfactant head group owing to the ion–dipole interaction between surfactant (SDS) and urea molecules. It is presumed that the positive dipoles on the hydrogens of NH_2 in urea molecule interact with the negatively charged SDS head group. However, in case of CTAB+urea system the interactions seems to have an opposite effect this may be due to the repulsive interaction between the positive charge on the CTAB head group and the positive dipoles of hydrogens of urea. No effect was observed on the mobility of metal cations in case of non–ionic surfactants (Brij–35 or Tx–100) added urea solvent systems. This observation further supports our above mentioned explanation regarding the interactive behavior of urea with ionic micelles.

Some of experimentally achieved separations of metal cations with mobile phases M_4, M_1, M_{13} and M_{14} have been listed in **Table–6.5** The mobile phase M_4 comprising aqueous micellar CTAB solution was found to facilitate analytically important separations of several metal cations by virtue of their variable mobility trends. For example Cr^{6+} or M_0^{6+} can be selectively separated from all other metal cations including Cr^{3+} because of their higher mobility compared to other metal cations in this mobile phase (M_4). The R_{AM} (R_T of upper spot–R_L of lower spot) values have been used to show the clear separation of resolved spots from the mixture. For separation, R_{AM} values should be positive. Higher the R_{AM} values, greater the difference between the separated spots and better is the separation. Separation of Cr^{6+} from Cr^{3+} is important from industrial point of view as

binary mixture of these cations play a significant role in several catalytic oxidation–reduction reactions.

Effect of pH on the mutual separation of Cr^{3+} and Cr^{6+} has been illustrated in **Table–6.6**. It is clear from this table that best separation of Cr^{6+} from Cr^{3+} from their mixture can be obtained in the pH range 2.3–5.7. At pH 1.0 Cr^{3+} gives slightly elongated spot and above pH 5.7 it precipitates out and could not be detected. Cr^{6+} gives constant R_F values over wider pH range (pH 1–8.5). However, in strong alkaline media (pH>8.5) precipitation occurs and its detection was not possible. The low R_F of Cr^{3+} shows its strong affinity to the stationary phase. It seems that Cr^{3+} exists as cationic species (63) in pH range 2.3–5.7 and retained by silica gel via cation exchange reaction

$$M^{3+}+3(-SiOH) \rightleftharpoons M(OSi-)_3+3H^+$$

Here, hydrolysis of silicon dioxide produces hydrous oxide surface group–SiOH, i.e., the silanol group. This results in the formation of silica surface a weakly acidic with $Ka=10^{-6}–10^{-8}$ (64). However, Cr^{6+} being anionic, is not exchanged and migrates with the solvent front.

The lowest possible detectable microgram amounts along with the dilution limits of metal cations achieved on HPTLC plates developed with M_4 is given in **Table–6.7.** The results of Table 6.7 indicate that the proposed method can be used for sensitive detection of Cr^{3+} and Cr^{6+}. Though, Cr^{6+} is self detected (sensitivity 0.8μg/spot), but its sensitivity can be raised about 80 times using $AgNO_3$ as detector (sensitivity 0.01μg/spot).

In addition to the qualitative analysis, a quantitative evaluation of the metal ions is often required to ascertain the level of the toxic metals in environmental sample. A relatively less accurate but simplest method for quantitation is based on the measurement of the size of the spot by drawing the outline of the spot on the piece of tracing paper. Therefore, an attempt was made to achieve semi–quantitative determination of chromium by measuring the spot area. A linear relation obtained when the amount of the

sample spotted was plotted against the area of the spot **(Fig–6.2)** follows the empirical equation Σ=km, where Σ is the area of the spot, m is the amount of the solute and K is a constant. The linearity is maintained upto 200μg/spot of Cr^{6+} or Cr^{3+}. At higher concentration, a positive deviation from linear law in both cases was observed. The accuracy and precision was around ± 12%.

The proposed method was successfully applied for identification and separation of heavy metal ions from spiked industrial wastewater sample and synthetically prepared alloys. The result presented in **Table–6.8** clearly demonstrate the applicability of the method for identification of Cr^{6+} as well as the mutual separation of coexisting Cr^{6+} and Cr^{3+} from industrial waste water sample and synthetically prepared alloys.

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Table–6.1

Mobility (R_F values) of metal ions developed with aqueous surfactants (CTAB, SDS, Brij-35 or TX-100) solutions above, near or below their critical micellar concentration (CMC) on HPTLC silica gel plates.

Mobile phase	R_F value*→				
	Cr^{6+}	Cr^{3+}	M_0^{6+}	Tl^{+}	Hg^{2+}
M_1	0.35T	0.02	0.83	0.71	0.12
M_2	0.95	0.00	0.92	0.04	0.05
M_3	0.95	0.00	0.93	0.03	0.03
M_4	0.96	0.02	0.93	0.04	0.66
M_5	0.95	0.03	0.87	0.04	0.02
M_6	0.94	0.00	0.77	0.02	0.05
M_7	0.96	0.00	0.92	0.02	0.03
M_8	0.96	0.02	0.94	0.03	0.04
M_9	0.94	0.00	0.95	0.04	0.02
M_{10}	0.95	0.03	0.92	0.03	0.02
M_{11}	0.97	0.00	0.92	0.03	0.1
M_{12}	0.95	0.00	0.93	0.02	0.4

* The R_F of Ni^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Bi^{3+}, Ag^{+}, VO^{2+}, UO_2^{2+}, Mn^{2+}, Fe^{3+}, Cu^{2+} and Ti^{4+} was in the range 0.00–0.10 in M_1–M_{12}.

Table–6.2

Mobility (R_F value) of some metal cations developed with mixed micellar solutions (cationic/anionic+non-ionic) on silica HPTLC plates.

Mobile phase	R_F value→				
	Cr^{6+}	Cr^{3+}	M_0^{6+}	Tl^{+}	Hg^{2+}
M_{13}	0.94	0.02	0.87	0.03	0.00
M_{14}	0.45T	0.03	0.92	0.43	0.06
M_{15}	0.92	0.00	0.93	0.02	0.00
M_{16}	0.56T	0.00	0.93	0.29	0.03

* R_F of Tl^{+} in 0.2 M SDS (M_1) is 0.71.

R_F of Ni^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Bi^{3+}, Ag^{+}, VO^{2+}, UO_2^{2+} Mn^{2+}, Al^{3+}, Fe^{3+}, Cu^{2+} and Ti^{4+} was in the range 0.00–0.80 in M_{13}–M_{16}.

Table–6.3

Mobility (R_F value) of metal ions developed with micellar solutions of CTAB, SDS, Brij-35 or TX-100 with added urea (1.0M) in 1:9 and 9:1 ratios by volume on silica HPTLC plates.

Mobile phase	R_F value→				
	Cr^{6+}	Cr^{3+}	M_0^{6+}	Tl^{+}	Hg^{2+}
M_{17}(a)	0.96	0.00	0.93	0.02	0.35
M_{17}(b)	0.93	0.00	0.95	0.04	0.65
M_{18}(a)	0.91	0.02	0.92	0.32	0.05
M_{18}(b)	0.95	0.03	0.93	0.57	0.04
M_{19}(a)	0.96	0.00	0.95	0.02	0.07
M_{19}(b)	0.94	0.02	0.92	0.03	0.06
M_{20}(a)	0.95	0.03	0.95	0.03	0.06
M_{20}(b)	0.95	0.00	0.96	0.02	0.05

R_F value of Ni^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Bi^{3+}, Ag^{+}, VO^{2+}, UO_2^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}, Cu^{2+} and Ti^{4+} was in the range of 0.00–0.09 in M_{17}(a)–M_{20}(b).

Table–6.4

Mobility (R_F value) of Cr^{6+} and Mo^{6+} on silica HPTLC plates developed with micellar solutions of CTAB, SDS, Brij–35 or TX–100 with added urea (2.0 M) in 1:9 or 9:1 ratios by volume.

Mobile phase	R_F value		
	Cr^{6+}	Cr^{3+}	$M_0{}^{6+}$
M_{21}(a)	(0.75)T	0.02	0.95
M_{21}(e)	0.93	0.03	0.96
M_{22}(a)	0.80	0.02	0.93
M_{22}(e)	0.92	0.00	0.92
M_{23}(a)	0.96	0.02	0.95
M_{23}(b)	0.92	0.02	0.96
M_{24}(a)	0.96	0.00	0.95
M_{24}(b)	0.96	0.00	0.93

Effect of urea (2.0M) on the mobility of Hg^{2+} and Tl^{+} has been represented in Figs 6.1(a) and (b).

Metal ions Ni^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Bi^{3+}, Ag^{+} VO^{2+}, $UO_2{}^{2+}$, Mn^{2+}, Al^{3+}, Fe^{3+}, Cu^{2+} and Ti^{4+} remained near the point of application (R_F = 0.00–0.10) in M_{21}(a)–M_{24}(b).

Table – 6.5

Separations achieved experimentally on silica HPTLC plates developed with mobile phases M_1, M_4, M_{13} or M_{14}.

Mobile phase	Separation	R_{AM} value
M_1	Tl^+-Fe^{3+}/Cu^{2+}/Ni^{2+}	0.54/0.55/0.30
	Tl^+-Co^{2+}/UO_2^{2+}/VO^{2+}	0.35/0.40/0.42
	Tl^+-Ag^+/Hg^{2+}/Bi^{3+}	0.45/0.30/0.40
	T^{l+}-Pb^{2+}/Cd^{2+}/Al^{3+}or Cr^{3+}	0.43/0.46/0.49
	Tl^+-Mn^{2+}	0.36
M_4	Cr^{6+}-Cr^{3+}/Fe^{3+}/Cu^{2+}	0.84
	Cr^{6+}-UO_2^{2+}/Ni^{2+}/Co^{2+}	0.75/0.80/0.81
	Cr^{6+}-VO^{2+}/Cd^{2+}or Ag^+/Pb^{2+}	0.70/0.82/0.80
	Hg^{2+}-Cr^{3+}/Fe^{3+}/Cu^{2+}	0.46/0.44/0.42
	Hg^{2+}-UO_2^{2+}/VO^{2+}/Ni^{2+}	0.44/0.36/0.32
	Hg^{2+}-CO^{2+}/Cd^{2+}or/Pb^{2+}/Ag^+	0.34/0.36/0.34
	Hg^{2+}-Tl^+/Bi^{3+}/Al^{3+}or Mn^{2+}	0.36/0.26/0.40
	Mo^{6+}-Cr^{3+}/Fe^{3+}orCu^{2+}/UO_2^{2+}or VO^{2+}	0.77/0.78/0.81
	Mo^{6+}-Ni^{2+}/Co^{2+}/Cd^{2+}	0.79/0.83/0.85
	Mo^{6+}-Pb^{2+}/Ag^+orTl^+/Bi^{3+}	0.88/0.86/0.77
	Mo^{6+}-Al^{3+}/Mn^{2+}	0.76/0.78
M_{13}	Hg^{2+}-Cr^{6+}/Mo^{6+}	0.86/0.80
M_{14}	Fe^{3+}-Tl^+-Mo^{6+}	0.26, 0.72*
	Cu^{2+}-Tl^+-Mo^{6+}	0.27, 0.73
	Cd^{2+}-Tl^+-Mo^{6+}	0.37, 0.70
	Ag^+-Tl^+-Mo^{6+}	0.40, 0.72
	Cr^{3+}-Tl^+-Mo^{6+}	0.26, 0.73
	Al^{3+}-Tl^+-Mo^{6+}	0.28, 0.77

* For the separation of three – component mixture with M_{14}, two R_{AM} values correspond to the separation of Fe^{3+}, Cu^{2+}, Cd^{2+}, Ag^+, Cr^{3+} or Al^{3+} from Tl^+ and Tl^+ from Mo^{6+} respectively. For example, for Fe^{3+}–Tl^+–Mo^{6+} separation, R_{AM} values for Fe^{3+}–Tl^+ and Tl^+–Mo^{6+} are 0.26 and 0.72 respectively.

Table–6.6

Effect of pH on the separation of Cr^{6+} and Cr^{3+} on silica HPTLC plates developed with M_4.

pH	Cr^{3+}	Cr^{6+}
1.0	0.16	0.96
2.3	0.03	0.96
3.4	0.02	0.94
5.7	0.02	0.95
7.0	ND	0.96
8.5	ND	0.95
9.9	ND	ND

a) ND refers to not detected

Table–6.7

Limit of detection of metal ions on silica HPTLC plates developed with aqueous micellar CTAB solution (M_4).

Metal ion	Limit of detection(μg)	Dilution limit [a]
Cr^{3+}	0.50	$1:2.0x10^4$
Cr^{6+}	0.8 (0.01)[b]	$1:1.25x10^4$

a) Dilution limit = 1: volume of test solution x 10^6 limit of detection.

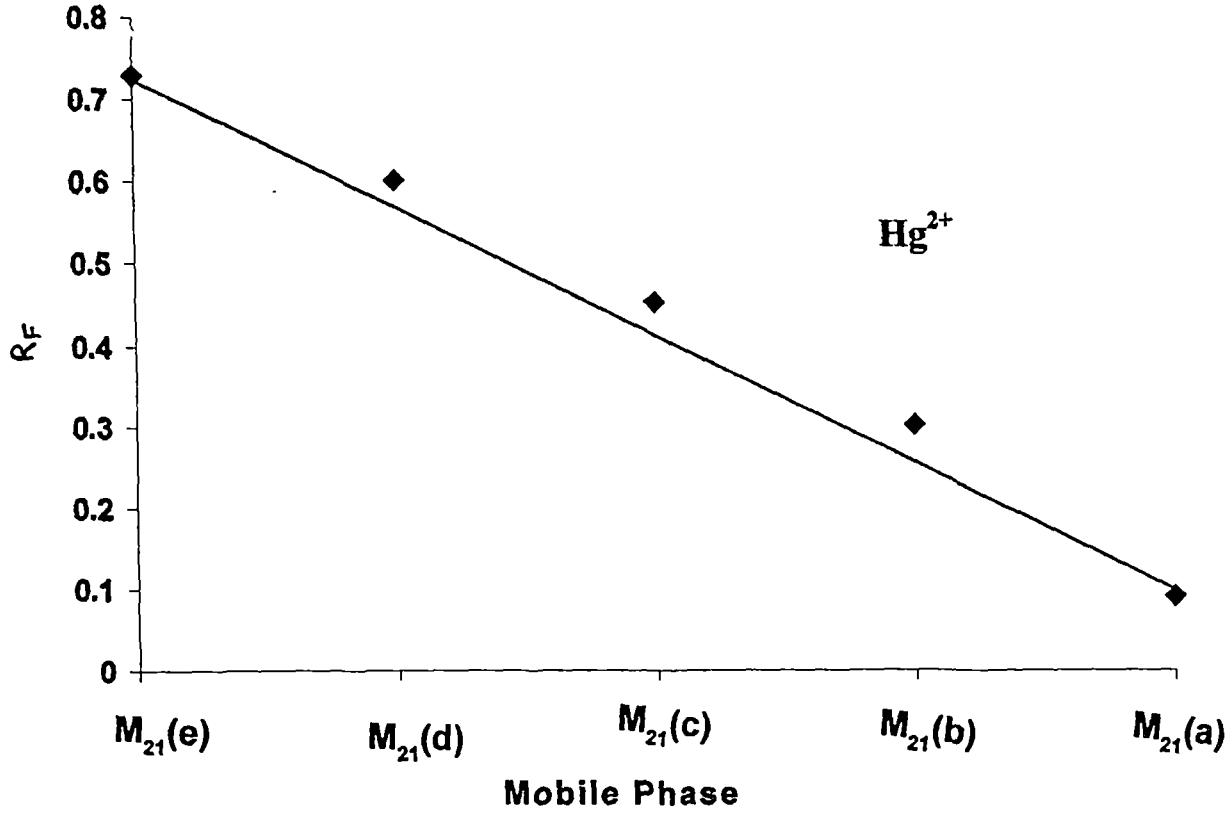
b) The value in parenthesis refer to the limit of detection of Cr^{6+} detected by using saturated alcoholic solution of $AgNO_3$.

Table–6.8

Separation of coexisting Cr^{3+} and Cr^{6+} from spiked industrial wastewater and synthetic alloys on silica HPTLC plates using mobile phase M_4.

Spiked/synthetic samples	Separation (R_F)	
	Cr^{3+}	Cr^{6+} (a)
Industrial wastewater	0.03	0.95
Cobalt alloy	0.03	0.90
Nichrome alloy	0.02	0.93

a) Detection of Cr^{6+} was done by saturated alcoholic solution of $AgNO_3$.



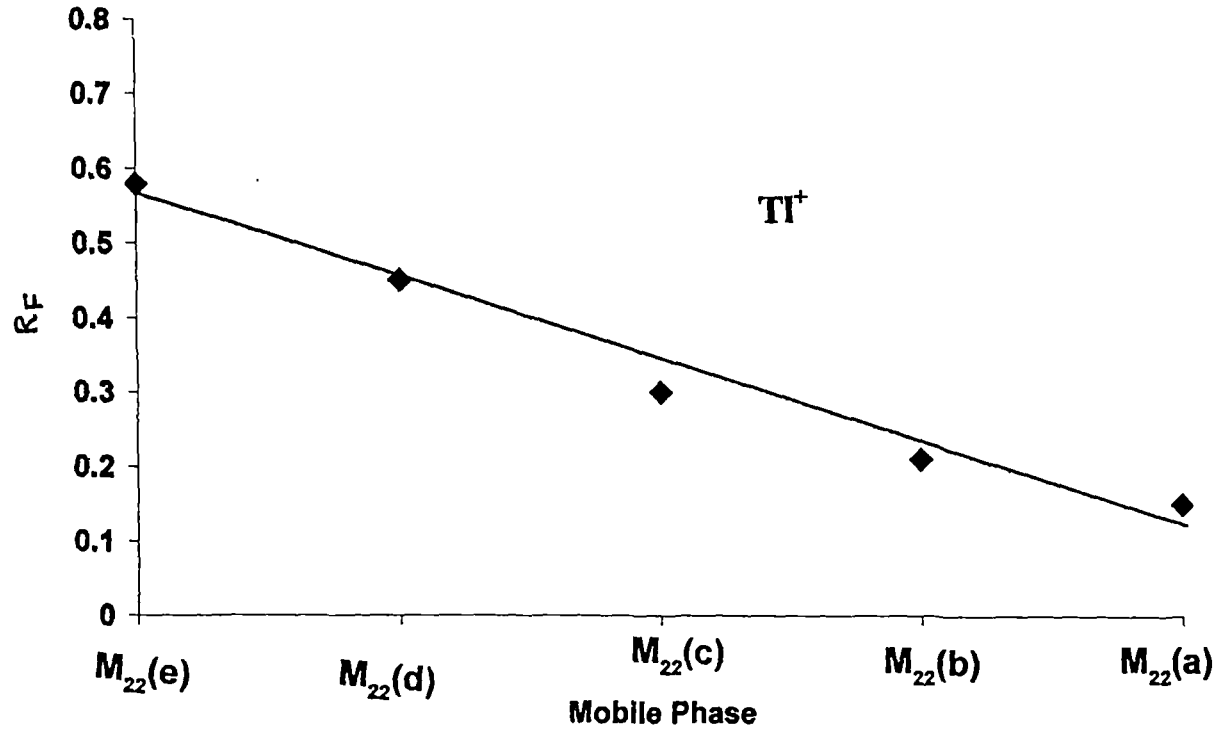
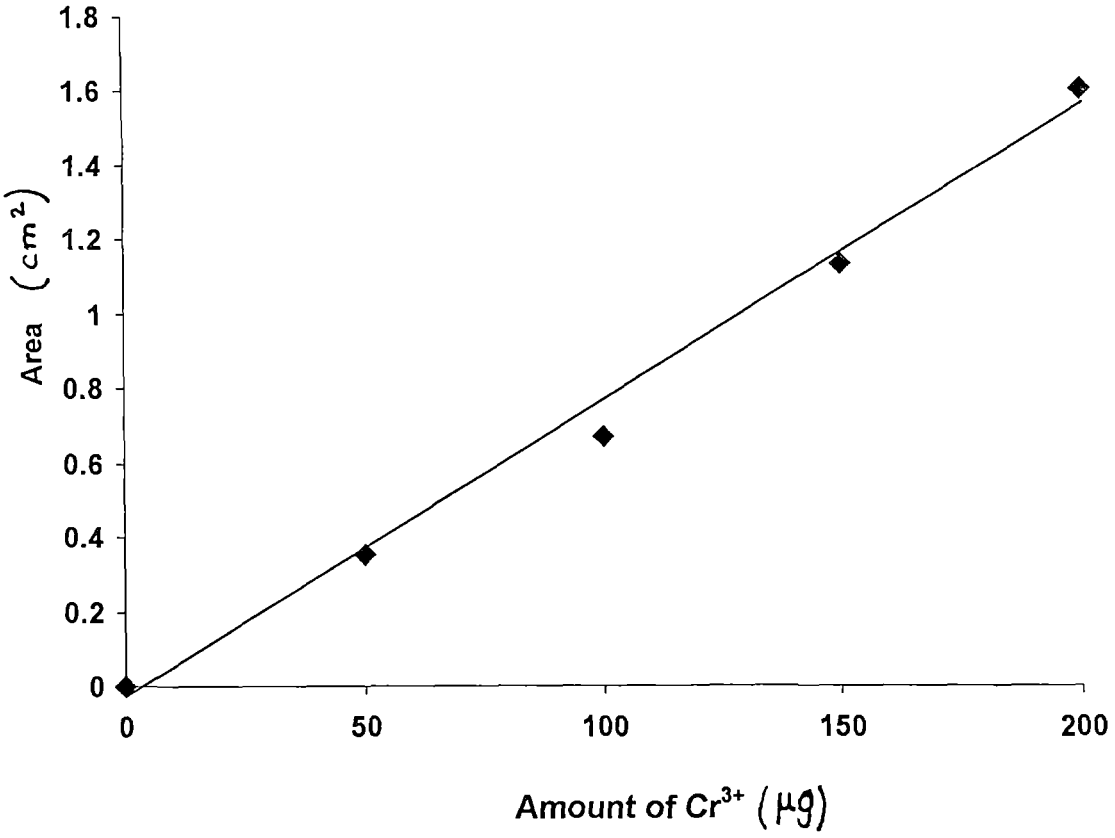


Fig 6.1 (a) (b) Plot of mobile phase Vs R_F values



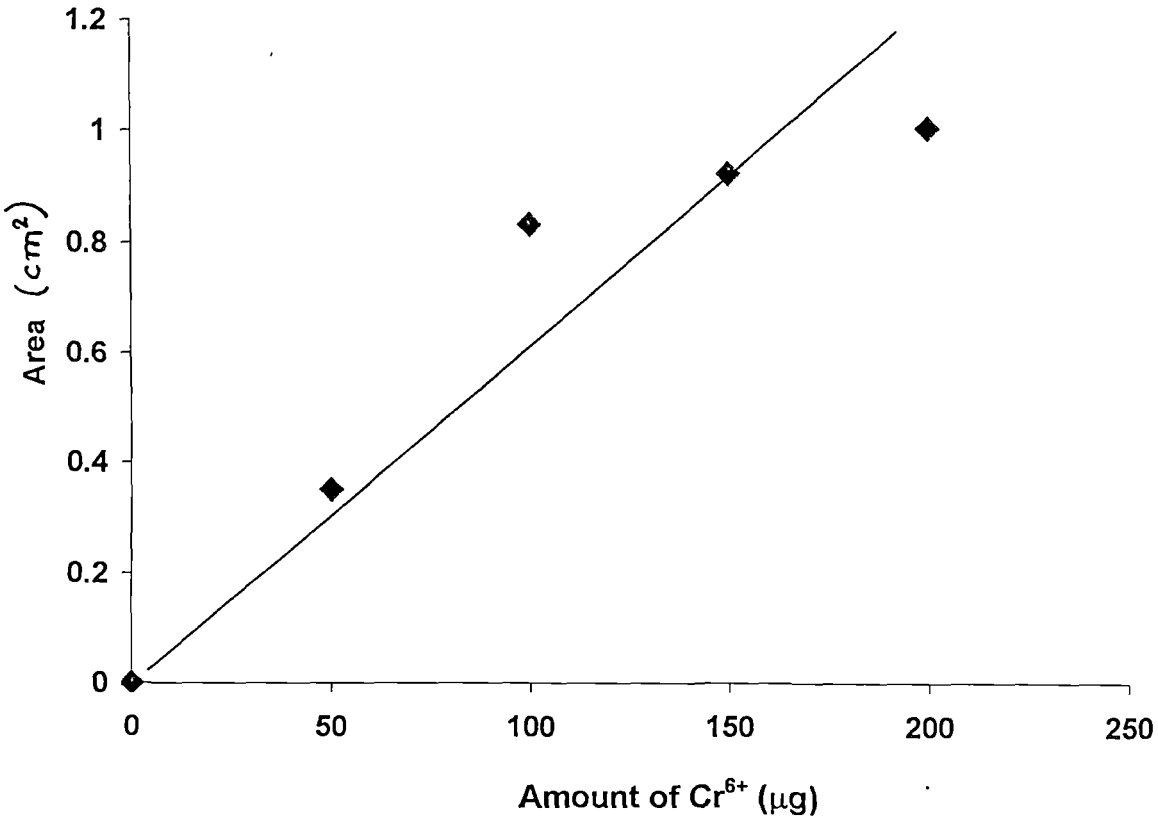


Fig 6.2 Plot of spot area Vs amount (µg) of Cr^{3+} or Cr^{6+}

CHAPTER-VII

Reversed-phase chromatography of metal cations, phenols and amines on silica layers impregnated with tributyl phosphate using surfactant-mediated mobile phase systems.

7.1 INTRODUCTION

Amongst the methods available for the analysis of inorganic ions, thin layer chromatography (TLC) is the most useful technique due to (i) low cost, (ii) minimal sample clean up, (iii) wide choice of mobile phases, (iv) flexibility in sample detection, (v) high sample loading capacity and (vi) easy handling. In 1985, Winschester (1) first combined the favourable features of high–molecular weight extractants with a chromatographic technique and separated a number of rare earth elements using di 2–ethyl hexyl phosphoric acid loaded on alumina as stationary phase and dilute HCl as the mobile phase. Since then, the technique of reversed–phase chromatography (or reversed–phase extraction chromatography) has become very popular. Several papers have been published (2–6) on reversed–phase thin layer chromatography (RPTLC) of metal ions using long–chain aliphatic amines, substituted quaternary ammonium salts, heterocyclic amines and neutral organophosphorous compounds as impregnants of the stationary phase. Solutions of strong monobasic acids or their alkali metal salts are generally selected as the mobile phase.

Tri–n–butyl phosphate (TBP), one of the most versatile extractants has received considerable attention in analytical separation chemistry because of its capability to extract most of the elements of the *Periodic Table* under suitable conditions of extraction. Since its first use in the separation of Th and V from impurities (7), TBP has been used extensively as extractant to accomplish analytical scale separations of actinides, lanthanides and Pt–group elements. However, its use for the separation of transition metals has been limited. From literature, layers of silica gel, polyvinyl chloride and cellulose impregnated with TBP have been used to separate various metal ions (8–10). The present work is aimed to utilize the analytical potential of TBP as impregnant in qualitative analysis of metal ions, phenols and amines. Separation and

identification of amines and phenols by TLC have received considerable attention during recent years because of their toxicological, pharmaceutical and industrial importance. Several sorbent phases including alumina, cellulose, polyacrylonitrile, silica gel, NH_2–modified silica gel and silica gel impregnated with inorganic metallic salts and organic nitro–compounds (11,16) have been used for the analysis of amines. Few studies have also been performed on TLC separation of amines via charge–transfer complexation with nitro–compounds (17,18). Some studies on the separation of phenols using various solvent systems (19–26) have been reported.

Surfactants are amphiphilic materials containing both non polar (hydrophobic) and polar (hydrophilic) groups. This dual character of the amphiphile leads to self-association or micellization of surfactants in water or similar solvents. Micellar solutions are transparent, non–volatile, inexpensive and relatively non–toxic. Though micelle structures have been extensively studied by physical chemists and biochemists, it is only recently that analytical chemists have realized that micellar systems can be advantageously utilized to chemical analysis (27). The apparent favourable features of using surfactant solutions as the mobile phase in liquid chromatography include (a) ability to solublize hydrophobic compounds, (b) highly selective partitioning of many solutes to micelles, (c) low cost and (d) the ability of changing of polarity of micellar mobile phase simply by changing the concentration of surfactant in solution. The unique separation possibilities of structurally similar solutes with micellar mobile phase arise because of the capability of micelles to solublize and bind a variety of solute molecules via hydrophobic, electrostatic and hydrogen bonding interactions.

Recently we have started a systematic study on the use of surfactant–mediated mobile phases in TLC separation of organic (28,29) and inorganic (30,31) substances. The present study was undertaken to determine the feasibility, effectiveness and advantages of using aqueous

surfactants as mobile phase for obtaining optimum conditions for identification and separation of amines and phenols (organic pollutants) and metal ions (inorganic pollutants) on TBP impregnated silica layers.

The studies reported so far include only the use of TBP impregnated layers in the analysis of either inorganic or organic substances separately. None of the studies refers to the use of TBP impregnated layers for the analysis of both organic and inorganic compounds in a particular solvent system. The present study deviates from TLC methods reported earlier as it includes the use of TBP impregnated layers in combination with surfactant (cationic, anionic or non–ionic) containing mobile phase systems for mutual separation of organic and inorganic compounds. The findings of our study may be helpful to remove inorganic impurities from organic compounds as well as the removal of organic impurities from inorganic (or metallic) compounds.

7.2 EXPERIMENTAL

Chemicals and Reagents:

Silica gel G (E. Merck, India), tributyl phosphate (Indian Drugs and Pharmaceuticals Ltd), sodium dodecyl sulphate, SDS (BDH, India); N,N,N–cetyltrimethyl ammonium bromide, CTAB (Central Drug House, India); Iso–octylphenoxypolyethoxy ethanol, Triton X–100 (termed as TX–100) and Brij–35 (polyoxyethylene dodecyl ether) both were obtained from Loba Chemical India and used as such without further purification. All other reagents used were also of Analytical Reagent grade.

Amines, Phenols and Metal Ions Studied:

Aliphatic and aromatic amines used in the present study include aniline (AL), o–chloroaniline (o–CAL), m–chloroaniline (m–CAL), p–chloroaniline (p–CAL), m–toluidine (m–TLD), p–toluidine (p–TLD),

diphenylamine (DPA), indole (ID), p–dimethylaminobenzaldehyde (p–DAB), L–tryptophan (L–TRYP), methylamine (MA), dimethylamine (DMA), trimethylamine (TMA), tributylamine (TBA), diethylamine (DEA) and triethylamine (TEA).

The phenolic derivatives used were, resorcinol (Rol), pyrogallol (Pol), phloroglucinol (PgL), o–cresol (o–Crol), m–cresol (m–Crol), p–cresol (p–Crol), m–nitrophenol (o–NPh), p–nitrophenol (p–NPh), o–nitrophenol (o–NPh), o–aminophenol (o–Aph), m–aminophenol (m–Aph), p–aminophenol (p–APh), orcinol (Ool) and pyrocatechol (p–Col).

Metal ions studied were, Fe^{3+}, Cu^{2+},Ni^{2+}, Co^{2+}, VO^{2+}, UO_2^{2+}, Zn^{2+}, Cd^{2+}, Ag^{+}, Pb^{2+}, Tl^{+}, Bi^{3+}, Hg^{2+},Mo^{6+}, Cr^{6+}, Cr^{3+} and Ti^{4+}.

Test Solutions:

The test solutions (1%) of all amines and phenols were prepared in methanol. Solutions (1%) of metal ions as their chloride, nitrate or sulphate salts were prepared in double distilled water with the addition of few drops of corresponding acids to prevent hydrolysis.

Detection:

The reagents used for detection of the cations were, 1% aqueous solution of potassium ferrocyanide for Cu^{2+}, UO_2^{2+}, VO^{2+}, Fe^{3+}, alcoholic alkaline solution of dimethylglyoxime (0.2%) for Ni^{2+} and Co^{2+} and 0.5% solution of dithizone in carbon tetrachloride for Cd^{2+}, Zn^{2+}, Ag^{+}, Tl^{+}, Bi^{3+}, Pb^{2+} and Hg^{2+}. For the detection of Cr^{6+} and Cr^{3+} saturated alcoholic solution of $AgNO_3$ and 1% methanolic solution of alizarin red 's' were used respectively. Aqueous solution of aluminon (1%) for Al^{3+}, 2.0M NaOH+ 30% H_2O_2 mixed in 1:1 ratio by volume for Mn^{2+} and 1% ferric chloride solution for Mo^{6+} were used. Amines and phenols were detected by exposing TLC plates to iodine vapours for 10 minutes.

Stationary Phase:

(a) Silica gel G

(b) Silica gel impregnated with 0.001M TBP in acetone.

Mobile phase:

The following solvent systems were used as mobile phase.

Symbol	Composition
M_1	Distilled water
M_2	0.1M SDS
M_3	0.01M SDS
M_4	0.0064 M SDS
M_5	0.00067 M SDS
M_6	0.01M CTAB
M_7	0.001M CTAB
M_8	0.00075M CTAB
M_9	0.000075M CTAB
M_{10}	0.03M TX–100
M_{11}	0.003M TX–100
M_{12}	0.002M TX–100
M_{13}	0.0002M TX–100
M_{14}	0.0012M Brij–35
M_{15}	0.00012M Brij–35
M_{16}	0.000083M Brij–35
M_{17}	0.00000832M Brij–35

CHROMATOGRAPHY

Preparation of TLC Plates

(a) Plain silica gel thin–layer plates

TLC plates were prepared by mixing silica gel G with demineralized water (DMW), in a 1:3 ratio. The resultant slurry was mechanically shaken for 5min and then it was coated onto glass plates with the help of a TLC applicator to give a layer of 0.25mm thickness. The plates were first air dried at room temperature and then activated by heating at 100°C for 1h. After activation, the plates were kept in air tight chamber until used.

(b) TBP impregnated TLC plates

Two methods were adopted for the preparation of impregnated silica gel plates (i) In the *precoating method* solution of the impregnant (TBP in acetone) was substituted for DMW in the slurry employed to coat the plates. For example, the slurry was made by mixing solution of 0.001M TBP (in acetone) with silica gel in the ratio of 3:1 and TLC plates were prepared by using the resultant slurry under the same experimental conditions as cited above in (a) for plain silica gel plates followed by activation at 100°C for 1h, (ii) *In post coating method* the activated silica gel plates were impregnated with desired concentration of TBP (0.001, 0.01, 0.1, 0.5, 1.0 or 2.0M) in acetone by dipping silica gel plates in solution of impregnant for a specific time period followed by drying of the plates at room temperature (30°C).

The post coating method was found to be suitable for the preparation of good quality silica gel plates and the plates prepared by this method were used during the present study.

Procedure

Thin layer chromatography was performed on TLC plates coated with unimpregnated and TBP impregnated silica gel layers in 24 x 6cm

glass jars. Approximately 10µl of standard test solutions were spotted separately on the TLC plate at 2cm from the lower edge of the plates with the help of a micropipette. The spot was allowed to dry and then the plates were developed in the desired solvent system by the ascending technique keeping the solvent ascent up to 10cm from the point of application at room temperature. After development, the plates were air dried at room temperature, and the position of amines or phenols on plates were located by exposing the TLC plates to iodine vapours for 10 min. Metal ions were detected by spraying the appropriate chromogenic reagent. R_F values were determined from the values of R_L (R_F of the leading front) and R_T (R_F of the trailing front).

$$R_F=(R_L+R_T)/2$$

Separation

The test solution (approx.10µL) containing two or more analytes (amine, phenol or metal ion) to be separated were spotted on TLC plates and the chromatography was performed using various mobile phases. The resolved spots were visualized by spraying suitable chromogenic reagent or by exposing TLC plates to iodine vapours for 10 min and the R_F values of the resolved spots of amines, phenols or metal ions were determined.

7.3 RESULTS AND DISCUSSION

The results of the present study have been summarized in **Tables 7.1–7.5** and **Figures 7.1 (a,b,c), 7.2 (a,b,c) and 7.3**. In order to find out optimum concentration of TBP impregnant, analytes (metal ions, amines and phenols) were chromatographed on silica layers impregnated with different concentrations (0.001M–2.0M) of TBP using distilled water (M_1) as developer. With the increase in the concentration of TBP, there occurs a considerable decrease in the R_F value of amines as can be seen from the representative plots presented in **Figs 7.1(a), (b) and (c)**. The decrease in R_F value (i.e. lowering in mobility) of amines may be attributed to strong partitioning of amines in TBP. In case of metal ions

and phenols similar trends i.e. decrease in R_F values with the increase in impregnant concentration was observed. However, at higher concentrations of impregnant TBP (>0.5M), tailed spots for certain metal ions and phenols appeared. The chromatography with silica plates impregnated at higher degree of TBP causes several problems including prolong development time, poor detection, lesser spot compactness, difficulty in drying of plates, poor adhesion of modified silica to glass surface etc. Therefore, TBP at concentration level of 0.001M was chosen for impregnation of silica gel plates, owing to the following advantages (i) lesser development time, (ii) detection clarity, (iii) formation of compact spots (iv) better durability of TLC plates.

Mobility of amines, phenols and metal ions was also examined on unimpregnated silica gel plates using DMW as eluent. The plots of ΔR_F (ΔR_F=R_F unimpregnated layers–R_F impregnated layers) have been shown in **Figs 7.2(a), (b) and (c)**. The positive ΔR_F values of amines (AL, o–CAL, p–TLD, p–NAL, MA, TMA, TEA or DPA), m–APh and all metal ions reveal enhanced selectivity of TBP impregnated silica layer towards these analytes. Contrary, the negative ΔR_F values of amines (m–AL, p–CAL, m–TLD, o–NAL, ID, p–DAB, L–TRYP, DMA, TBA or DEA) and all phenols show higher selectivity of plain (or unimpregnated) silica layers towards these analytes. However, better chromatographic performance on TBP impregnated plates was observed in respect of clarity of detection, compactness of spots and improved separations. For example, binary separation of p–DAB from ID or TRYP; of Cd^{2+} from Zn^{2+} or Pb^{2+}; and of m–APh from m–NPh etc. could be achieved only on TBP impregnated layers.

The mobility of amines, phenols and metal ions was also studied using aqueous surfactant (anionic, cationic or non–ionic) solutions as mobile phase keeping the concentration of surfactant (anionic, cationic or non–ionic) at about twelve times higher or lower and 1.2 times higher or

lower of their critical micellar concentration (CMC) value. The CMC values, given in parenthesis of SDS, CTAB, TX–100 and Brij–35 in water are SDS (0.008M), CTAB (0.0009M), TX–100 (0.0028M) and Brij35 (0.0001M) respectively. The mobility trends of amines, phenols and metal ions observed on (0.001M) TBP impregnated silica layers developed with different concentrations of anionic, cationic or non–ionic surfactants are discussed below:

Chromatography with SDS

The mobility of amines, phenols and metal ions was examined with aqueous solution of anionic SDS surfactant. Though some of amines (e.g. AL, m–CAL, p–CAL, m–TLD or p–TLD), phenols (Rol, Pol, PGL, m–NPh, p–NPh, Ool or PCol) and metal ions (Ni^{2+}, Cd^{2+}, Mo^{6+} or Mn^{2+}) were found to exhibit high mobilities but due to lack of differential migration and appearance of elongated spots good separation of coexisting metal ions, amines or phenols could not be achieved with aqueous SDS mobile phases (M_2–M_5).

Chromatography with CTAB

The results obtained with CTAB are listed in **Table 7.1**. From the available data as listed in Table 7.1, following mobility trends were noticed.

Amines

(i) Tailed spots (R_L–R_T>0.3) for p–DAB were observed with mobile phases containing 0.01–0.001M CTAB (M_6, M_7). However, well formed compact spots of p–DAB were realized with mobile phase M_8 or M_9 containing much lower concentration of CTAB compared to M_6 or M_7. In case of all other amines compact spots appeared irrespective of the concentration of CTAB (M_6–M_9).

(ii) O-AL remained not detected, P–NAL gave tailed spots and DPA either remained near the point of application (R_F=0.02–0.07) or gave tailed spot at all concentrations of CTAB (M_6–M_9) irrespective of the fact whether CTAB used was above or below its CMC value.

Phenols

All phenols with the exception of m–Crol, p–Crol or o–APh (R_F=0.00) showed high and more or less constant mobility (R_F=0.70–0.95) irrespective of the concentration of cationic surfactant (above or below CMC) in the mobile phase.

Metal Ions

(i) Ni^{2+} showed high mobility (R_F values = 0.72–0.82) at all concentration levels of CTAB (M_6–M_9). However, tailed spot of Ni^{2+} was visualized with non-micellar solution of CTAB (M_9) containing surfactant concentration about 12 times lower the CMC or CTAB.

(ii) Co^{2+} or Mn^{2+} moved near the middle of the chromatoplate (R_F=0.47–0.6) whereas Cr^{6+} and Mo^{6+} exhibited high mobility (R_F=0.78–0.97) with all mobile phases (M_6–M_9).

(iii) Cd^{2+} showed little mobility (R_F = 0.25–0.32) whereas Hg^{2+}, Ag^{+}, Bi^{3+} or Ti^{4+}, showed tailed spots at all concentration levels of CTAB (M_6–M_9).

Conclusion (SDS vs CTAB)

Aqueous micellar CTAB was found to be best for achieving some good separations of metal ions and phenols. For example; Cr^{3+}–Cr^{6+}, Fe^{3+}–Co^{2+}–Cr^{6+}, VO^{2+}–Mn^{2+}–Mo^{6+}, o–Crol–m–Crol, or m–APh–o–APh etc. were successfully separated from their mixtures. These separations could not be achieved with anionic surfactant SDS because well resolved

spots could not be visualized with aqueous micellar/non micellar solution of SDS used as mobile phase (M_2–M_5). Thus, CTAB is better eluent than SDS for achieving separations of metal ions and phenols as it induces differential migration, yields more compact spots and superior detection clarity. This result is in consonance with our earlier studies on TLC of metal ions (32).

Chromatography with TX–100

With different concentrations of non–ionic surfactant, TX–100 used as mobile phase (M_{10}–M_{13}), some of amines (AL, m–CAL, p–CAL, m–TLD, p–TLD, o–NAL or ID), phenols (Rol, Pol, PGL, m–NPh, p–NPh, m–APh, p–APh, Ool or PCol) and metal ions (Cr^{6+}, Mo^{6+}, Ni^{2+}, or Co^{2+}), moved near the solvent front (R_F=0.70–0.92) but due to appearance of elongated spots and lack of differential migration, separations could not be achieved experimentally. Interestingly some important ternary separations of amines and phenols e.g. o-APh–m-APh–p-APh, DPA–p-DAB–L-TRYP or DPA–ID–L-TRYP etc. appeared possible with M_{12}, therefore the aqueous solution of TX–100 (M_{12}) with some modifications might prove to be useful for achieving important separations of amines and phenols.

Chromatography with Brij–35

The results obtained with Brij–35 containing mobile phase systems (M_{14}–M_{17}) have been encapsulated in **Table 7.2**. From the data listed in Table 7.2 following conclusions regarding the mobility trends of amines, phenols and metal ions may be drawn:

Amines

(i) AL, m–CAL, p–CAL, m–TLD, p–TLD, o–NAL, ID, L–TRYP showed high mobility (RF=0.60–0.89) and p–DAB moved near the middle of the chromatoplate (R_F=0.45–0.56) at all concentration levels of Brij–35 (M_{14}–M_{17}). DPA

remained near the point of application (R_F = 0.02) producing a highly compact spot with M_{14} but it gave tailed spots with M_{15}–M_{17}). Thus, DPA can be separated from other amines using M_{14} as mobile phase.

Phenols

(i) Rol, Pol, PGL, m–NPh, m-APh, p–APh, Ool or PCol exhibited high mobilities (0.72–0.97) at all concentration levels of Brij-35 (M_{14}-M_{17}). With aqueous micellar solution of Brij–35 (M_{14} and M_{15}), o–Crol showed high R_F values of 0.82 and 0.80 respectively. But it remained near the point of application with non–micellar aqueous solution of Brij–35 (M_{16} or M_{17}). Thus, the mobility of o-Crol is strongly controlled by the concentration of Brij in the aqueous phase.

(ii) O–APh remained at the point of application (R_F=0.00) at all concentration levels of Brij–35 with the exception of mobile phase M_{15} where tailed spot of o–APh was realized.

Metal Ions

(i) .Ni^{2+}, Co^{2+}, Cr^{6+} or Mo^{6+} exhibited high mobility (R_F=0.68–0.97) at all concentration levels of Brij-35 (M_{14}-M_{17}). Cd^{2+} showed R_F value of 0.72 and 0.74 with M_{14} and M_{17} respectively. However, Cd^{2+} exhibited comparatively lower mobility with M_{15} and M_{16} giving R_F values of 0.47 and 0.34 respectively.

(ii) Tl^{+}, Bi^{3+} or Hg^{2+} gave tailed spots and Zn^{2+} remained near the point of application (R_F = 0.07-0.10) at all concentrations of Brij–35, with the exception of mobile phase M_{14} where tailed spot of Zn^{2+} was visualized.

Conclusion (TX–100 vs Brij–35)

Among non–ionic surfactants, Brij–35 proved to be a better eluent for achieving certain important binary separations of amines by virtue of differential migration of p–DAB, ID and L–TRYP. These separations could not be obtained when Brij–35 was substituted with TX–100 due to the formation of elongated spots of amines.

Some of the important separations of amines, phenols and metal ions, achieved experimentally, have been listed in **Table 7.3**. From where it is apparent that best TLC system for separation of amines constitutes silica layers impregnated with TBP (0.001M) as stationary phase and aqueous solution of Brij–35 (M_{17}) as mobile phase. With this chromatographic system, p–DAB (R_F=0.47), was successfully separated from ID (R_F=0.71) or L-TRYP (R_F=0.77) from their binary mixtures. Separation of p–DAB from indole is particularly important because p–DAB forms coloured products with ID in solution (33). Furthermore p-DAB and ID react with 2,4 dinitrotoluene (DNT) in the solid state to produce yellow products (34) and hence the presence of p–DAB hampers the detection of ID with DNT. Separation of p-DAB from ID constituents the separation of tertiary amine (p–DAB) from secondary amine (ID). It is interesting to note from this Table that N–compounds containing two aryl groups (ID and TRYP) move faster than p–DAB which has only one aryl group. TLC system comprising aqueous micellar solution of cationic surfactant CTAB (M_6) and TBP (0.001M) impregnated silica layers is best for achieving good separations of coexisting metal ions and phenols. Some important separations worth mentioning with this TLC system include: Cr^{3+}–Cr^{6+}, Fe^{3+}–Mn^{2+}–Cr^{6+}, VO^{2+}–Mn^{2+}–Cr^{6+}, o-Crol–m-Crol, p-APh–o-APh etc. Ternary separation of Fe^{3+} or VO^{2+} from Mn^{2+} and Cr^{6+} is important as these metal cations belong to the first transition series of the periodic table, and have similar physico-chemical properties.

In order to widen the applicability of proposed TLC method, the mutual separations of amines or phenols in the presence of metal cations and the separations of metal cations in the presence of amines or phenols have been successfully attended. The results so obtained have been summarized in **Tables 7.4 and 7.5**.

Inorganic ions (e.g. Fe^{3+}, Cu^{2+} or Ni^{2+} etc.) are used as catalyst in various chemical reactions hence, they may be present in the form of impurities in organic compounds. Similarly, organometallic compounds may contain trace amounts of organic impurities. It is evident from Tables 7.4 and 7.5 that coexisting metal ions could be separated in the presence of organic (amino or phenolic) impurities, likewise amines or phenols could be separated in the presence of inorganic impurities.

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Table–7.1

R_F values of amines, phenols and metal ions on TBP (0.001M) impregnated silica layers developed with aqueous solutions of cationic surfactant CTAB (M_6–M_9).

M_6						M_7					
Amines	R_F value	phenols	R_F value	Metal ions	R_F value	Amines	R_F value	Phenols	R_F value	Metal ions	R_F value
AL	0.84	Rol	0.88	Ni^{2+}	0.72	AL	0.71	Rol	0.81	Ni^{2+}	0.72
m–CAL	0.75	Pol	0.96	Co^{2+}	0.55	m–CAL	0.75	Pol	0.95	Co^{2+}	0.55
p–CAL	0.76	PGL	0.87	Cd^{2+}	0.28	p–CAL	0.72	PGL	0.79	Cd^{2+}	0.32
m–TLD	0.77	O–Crol	0.78	Ag^{+}	0.15T	m–TLD	0.74	o–Crol	0.79	Ag^{+}	0.15T
p–TLD	0.72	m–NPh	0.86	Tl^{+}	0.17T	p–TLD	0.71	m–NPh	0.83	Tl^{+}	0.15T
o–NAL	0.70	p–NPh	0.81	Bi^{3+}	0.16T	o–NAL	0.57	p–NPh	0.88	Bi^{3+}	0.17T
p–NAL	0.40T	m–APh	0.72	Hg^{2+}	0.36T	o-NAL	0.44T	p–APh	0.80	Hg^{2+}	0.20T
DPA	0.02	p–APh	0.88	Cr^{6+}	0.97	DPA	0.07	p–APh	0.88	Cr^{6+}	0.97
ID	0.57	Ool	0.83	Mo^{6+}	0.85	ID	0.60	Ool	0.79	Mo^{6+}	0.92
P–DAB	0.26T	Pcol	0.76	Mn^{2+}	0.48	p-DAB	0.27T	PCol	0.75	Mn^{2+}	0.49
L–TRYP	0.77					L–TRYP	0.84				

Continue

M_8						M_9					
a)		b)		c)							
Amines	R_F value	phenols	R_F value	Metal ions	R_F value	Amines	R_F value	Phenols	R_F value	Metal ions	R_F value
AL	0.86	Rol	0.90	Ni^{2+}	0.82	AL	0.82	Rol	0.92	Ni^{2+}	0.71T[d]
m–CAL	0.90	Pol	0.80	Co^{2+}	0.60	m–CAL	0.91	Pol	0.91	Co^{2+}	0.48
p–CAL	0.72	PGL	0.85	Cd^{2+}	0.30	p–CAL	0.81	PGL	0.88	Cd^{2+}	0.25
m–TLD	0.73	O–Crol	0.89	Ag^{+}	0.16T	m–TLD	0.75	o–Crol	0.84	Ag^{+}	0.15T
p–TLD	0.80	m–NPh	0.89	Tl^{+}	0.17T	p–TLD	0.82	m–NPh	0.83	Tl^{+}	0.15T
o–NAL	0.63	P–NPh	0.88	Bi^{3+}	0.15T	o–NAL	0.61	p–NPh	0.86	Bi^{3+}	0.18T
p–NAL	0.37T	m–Aph	0.70	Hg^{2+}	0.55T	p–NAL	0.45T	m–APh	0.84	Hg^{2+}	0.62T
DPA	0.25T	P–Aph	0.90	Cr^{6+}	0.96	DPA	0.25T	p–APh	0.92	Cr^{6+}	0.78
ID	0.67	Ool	0.82	Mo^{6+}	0.91	ID	0.91	Ool	0.78	Mo^{6+}	0.89
p–DAB	0.56	Pcol	0.70	Mn^{2+}	0.47	p-DAB	0.62	PCol	0.78	Mn^{2+}	0.51
L–TRYP	0.84					L–TRYP	0.91				

a) o–CAL remained not detected, MA, DMA, TMA, DEA or TEA (aliphatic amines) remained near the point of application (R_F=0.02–0.11)

b) m–Crol, p–Crol or o–APh remained at the point of application (R_F=0.00)

c) Fe^{3+},Cu^{2+} UO_2^{2+}, VO^{2+}, Zn^{2+}, Pb^{2+}, Al^{3+}, Cr^{3+} or Ti^{4+} remained near the point of application (R_F=0.02–0.06)

d) T refers to tailed spot (R_L–R_T>0.30)

Table–7.2

R_F values of amines, phenols and metal ions on TBP (0.001M) impregnated silica layers developed with aqueous solutions of Brij–35 (M_{14}–M_{17})

M_{14}						M_{15}					
Amines	R_F value	Phenols	R_F value	Metal ions	R_F value	Amines	R_F value	Phenols	R_F value	Metal ions	R_F value
AL	0.85	Rol	0.94	Ni^{2+}	0.86	AL	0.82	Rol	0.92	Ni^{2+}	0.73
m–CAL	0.69	Pol	0.95	Co^{2+}	0.85	m–CAL	0.62	Pol	0.94	Co^{2+}	0.82
p–CAL	0.65	PGL	0.97	Cd^{2+}	0.72	p–CAL	0.60	PGL	0.96	Cd^{2+}	0.47
m–TLD	0.75	o–Crol	0.82	Zn^{2+}	0.16T	m–TLD	0.65	o–Crol	0.80	Zn^{2+}	0.07
p–TLD	0.72	m–NPh	0.82	Tl^{+}	0.20T	p–TLD	0.62	m–NPh	0.82	Tl^{+}	0.17T
o–NAL	0.67	p–NPh	0.89	Bi^{3+}	0.15T	o–NAL	0.60	p–NPh	0.80	Bi^{3+}	0.17T
p–NAL	0.40T	m–Aph	0.00	Hg^{2+}	0.70T	p-NAL	0.35T	o–APh	0.30T	Hg^{2+}	0.60T
DPA	0.02	p–Aph	0.83	Cr^{6+}	0.95	DPA	0.27T	m–APh	0.72	Cr^{6+}	0.97
ID	0.83	Ool	0.75	Mo^{6+}	0.94	ID	0.81	p–APh	0.81	Mo^{6+}	0.97
p–DAB	0.52	Ool	0.81	Mn^{2+}	0.88	p-DAB	0.56	Ool	0.84	Mn^{2+}	0.72
L–TRYP	0.89	Pcol	0.85			L–TRYP	0.84	PCol	0.90		

Continue

M_{16}						M_{17}					
a)		b)		c)							
Amines	R_F value	phenols	R_F value	Metal ions	R_F value	Amines	R_F value	Phenols	R_F value	Metal ions	R_F value
AL	0.79	Rol	0.92	Ni^{2+}	0.82	AL	0.82	Rol	0.92	Ni^{2+}	0.92
m–CAL	0.72	Pol	0.90	Co^{2+}	0.68	m–CAL	0.75	Pol	0.95	Co^{2+}	0.92
P–CAL	0.70	PGL	0.97	Cd^{2+}	0.34	p–CAL	0.76	PGL	0.94	Cd^{2+}	0.74
m–TLD	0.79	o–Crol	0.00	Zn^{2+}	0.07	m–TLD	0.69	o–Crol	0.02	Zn^{2+}	0.10
p–TLD	0.82	m–NPh	0.79	Tl^{+}	0.16T	p–TLD	0.72	m–NPh	0.80	Tl^{+}	0.16T
o–NAL	0.69	p–NPh	0.86	Bi^{3+}	0.17T	o–NAL	0.65	p–NPh	0.87	Bi^{3+}	0.18T
p–NAL	0.52T	m–Aph	0.00	Hg^{2+}	0.17T	p–NAL	0.41T	m–APh	0.00	Hg^{2+}	0.36T
DPA	0.17T	p–Aph	0.73	Cr^{6+}	0.92	DPA	0.25T	p–APh	0.82	Cr^{6+}	0.97
ID	0.75	Ool	0.85	Mo^{6+}	0.90	ID	0.75	p–APh	0.84	Mo^{6+}	0.94
p–DAB	0.45	Ool	0.83	Mn^{2+}	0.65	p–DAB	0.48	Ool	0.80	Mn^{2+}	0.60
L–TRYP	0.84	Pcol	0.94			L–TRYP	0.89	PCol	0.95		

a) o–CAL remained not detected, MA, DMA, TMA, DEA or TEA (aliphatic amines) remained near the point of application (R_F=0.02–0.12)

b) m–Crol, p–Crol remained at the point of application (R_F=0.00)

c) Fe^{3+},Cu^{2+} VO_2^{2+}, Ag^{+}, Pb^{2+}, Al^{3+}, Cr^{3+} or Ti^{4+} remained near the point of application (R_F=0.02–0.11)

Table-7.3

Some important separations of amines, phenols and metal ions on TBP (0.001M) impregnated silica layers using Mobile phase M_6 or M_{17}

Analyte	Mobile phase	Separations (R_F)
Amines	M_{17}	p–DAB (0.47) –ID(0.71)*, p–DAB (0.48) – p–TLD(0.69), p–DAB (0.45) –m–CAL(0.72), p–DAB (0.44) –p–CAL(0.75), p–DAB(0.46) –L–TRYP(0.77)*, o–NAL(0.63) –AL(0.80)
Phenols	M_6	o–Crol (0.75) –m–Crol (0.00)*, o–Crol (0.76) –p–Crol (0.00), m–APh (0.72) –o–Aph (0.00), p–APh (0.85)–o–APh (0.00), m–Aph (0.70) –m–Crol (0.00), o–Crol (0.74) –O–Aph (0.00), p–APh (0.84) –p–Crol (0.00), Rol (0.85)-m–Crol (0.00) or p–Crol (0.00) or o–APh (0.00), Pol (0.93)–p–Crol (0.00) or m–Crol (0.00) or o–APh (0.00), PGL(0.86) –m–Crol(0.00) or p–Crol (0.00) or o-APh(0.00
Metal ions	M_6	Cr^{6+}(0.96) –Cr^{3+}(0.03)*, VO^{2+}(0.2) –Mn^{2+}(0.45) –Cr^{6+}(0.96)* Fe^{3+}(0.03) –Co^{2+}(0.51) –Cr^{6+}(0.96), Fe^{3+}(0.02) –Mn^{2+}(0.41) – Cr^{6+}(0.97)*, VO^{2+}(0.02)–Mn^{2+}(0.49)-Mo^{6+}(0.92)

* Traced pictures of the chromatograms has been provided for clear illustration of the achieved separations (Fig 7.3)

Table–7.4

Separation of metal ions in the presence of amines or phenolic impurities developed with mobile phase M_6 on TBP (0.001) impregnated silica plates.

Impurities	Separations (R_F)
AL	Cr^{3+}(0.03)–Cr^{6+}(0.82), VO^{2+}(0.04)–Mn^{2+}(0.62) –Mo^{6+}(0.86), Fe^{3+}(0.02) –Mn^{2+}(0.60) –Cr^{6+}(0.82), Fe^{3+}(0.04) –Co^{2+}(0.49) –Cr^{6+}(0.83), VO^{2+}(0.02) –Mn^{2+}(0.45) –Cr^{6+} (0.80)
o–CAL	Cr^{3+}(0.02) –Cr^{6+}(0.81), VO^{2+}(0.02) –Mn^{2+}(0.59) –Mo^{6+}(0.85), Fe^{3+}(0.02) –Mn^{2+}(0.57) –Cr^{6+}(0.84), Fe^{3+}(0.02) –Co^{2+}(0.52) –Cr^{6+}(0.84), VO^{2+}(0.03) –Mn^{2+}(0.48) –Cr^{6+}(0.82).
m- CAL	Cr^{3+} (0.03)- Cr^{6+} (0.82), VO^{2+} (0.03)- Mn^{2+} (0.69)- Mo^{6+} (0.83), Fe^{3+} (0.03)- Mn^{2+} (0.55)- Cr^{6+} (0.83), Fe^{3+} (0.02)- Co^{2+} (0.50)- Cr^{6+} (0.85), VO^{2+} (0.02)- Mn^{2+} (0.46)- Cr^{6+} (0.83).
m–TLD	Cr^{3+}(0.04) –Cr^{6+}(0.87), VO^{2+}(0.02) –Mn^{2+}(0.58) –Mo^{6+}(0.84), Fe^{3+}(0.00) –Mn^{2+}(0.55) –Cr^{6+}(0.83), Fe^{3+}(0.02) –Co^{2+}(0.50) –Cr^{6+}(0.82), VO^{2+}(0.02) –Mn^{2+}(0.45) –Cr^{6+}(0.82)
p–NAL	Cr^{3+}(0.02) –Cr^{6+}(0.85), VO^{2+}(0.02) –Mn^{2+}(0.56) –Mo^{6+}(0.83), Fe^{3+}(0.04) –Mn^{2+}(0.52) –Cr^{6+}(0.82), Fe^{3+}(0.04) –Co^{2+}(0.40) –Cr^{6+}(0.75), VO^{2+}(0.02) –Mn^{2+}(0.42) –Cr^{6+}(0.76)
DMA	Cr^{3+}(0.02) –Cr^{6+}(0.81), VO^{2+}(0.03) –Mn^{2+}(0.82), Fe^{3+}(0.03) –Mn^{2+}(0.57) –Cr^{6+}(0.79), Fe^{3+}(0.00) –Co^{2+}(0.45) –Cr^{6+}(0.77), VO^{2+}(0.22) –Mn^{2+}(0.45) –Cr^{6+}(0.80)
PCol	Cr^{3+}(0.05) –Cr^{6+}(0.89), VO^{2+}(0.03) –Mn^{2+}(0.50) –Mo^{6+}(0.85), Fe^{3+}(0.05) –Mn^{2+}(0.49) –Cr^{6+}(0.85), Fe^{3+}(0.05) –Co^{2+}(0.49) –Cr^{6+}(0.84), VO^{2+}(0.04) –Mn^{2+}(0.47) –Cr^{6+}(0.82).
PGL	Cr^{3+}(0.04) –Cr^{6+}(0.90), VO^{2+}(0.05) –Mn^{2+}(0.50) –Cr^{6+}(0.86), VO^{2+}(0.02) –Mn^{2+}(0.52) –Mo^{6+}(0.89), Fe^{3+}(0.04) –Mn^{2+}(0.54) –Cr^{6+}(0.84), Fe3++$^{+}$(0.02) –Co^{2+}(0.52) –Cr^{6+}(0.82).

Table–7.5

Separation of amines and phenols in the presence of inorganic impurities developed with mobile phases M_6 or M_{17} on 0.001M TBP impregnated silica layers.

Impurities	Separation (R_F)
Fe^{3+}	p-DAB(0.45) –ID(0.70), p–DAB(0.44) –L-TRYP(0.76), o-Crol(0.74) –m-Crol(0.00), o-Crol(0.74) –p-Crol(0.00), m-Aph(0.70)/p-APh(0.83) –o-APh(0.00).
Cu^{2+}	p-DAB(0.46) –ID(0.71), p-DAB(0.45) –L-TRYP(0.75), o-Crol(0.73) –m-Crol(0.00), o-Crol (0.75) –p-Crol(0.00), m-Aph(0.73)/p-APh(0.84) –o-APh(0.02).
Ni^{2+}	p-DAB(0.47) –ID(0.72), p-DAB(0.45) –L-TRYP(0.74), o-Crol(0.72) –m-Crol(0.00), o-Crol(0.76) –p-Crol(0.02), m-APh(0.72)/p-APh(0.82) –o-APh(0.00).
Co^{2+}	p-DAB(0.46) –ID(0.71), p-DAB(0.45) –L-TRYP(0.74), o-Crol(0.73) –m-Crol(0.00), o–Crol(0.76) –p-Crol(0.00), m-Aph(0.73)/p-APh(0.83)–o-APh(0.00).
UO_2^{2+}	p-DAB(0.48) –ID(0.70), p-DAB(0.46) –L-TRYP(0.75), o-Crol(0.73) –m-Crol(0.00), o-Crol(0.75) –p-Crol(0.00), m-APh(0.72)/p-APh(0.82) –o-APh(0.00).

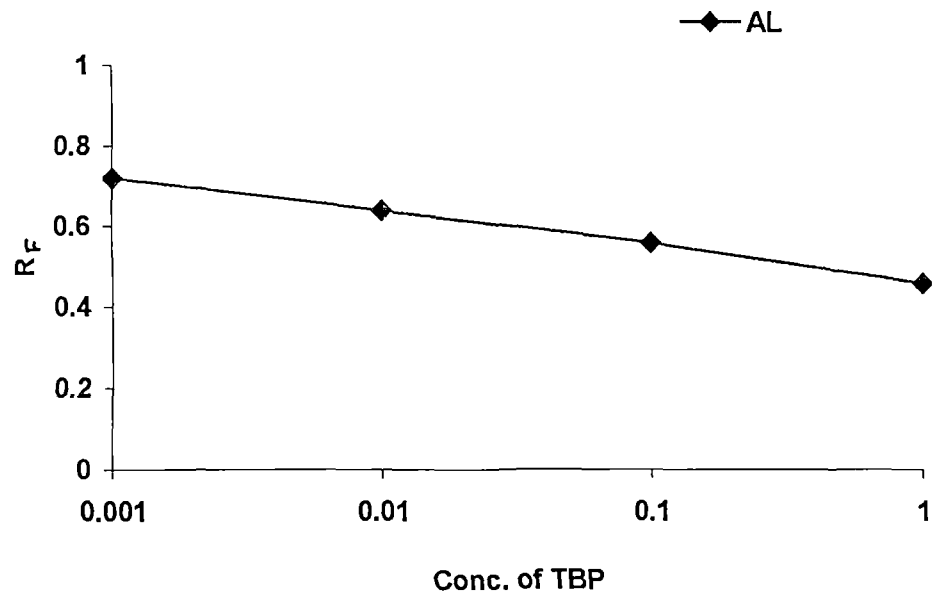

Fig.7.1(a) Plot of R_F V/s Conc. of TBP

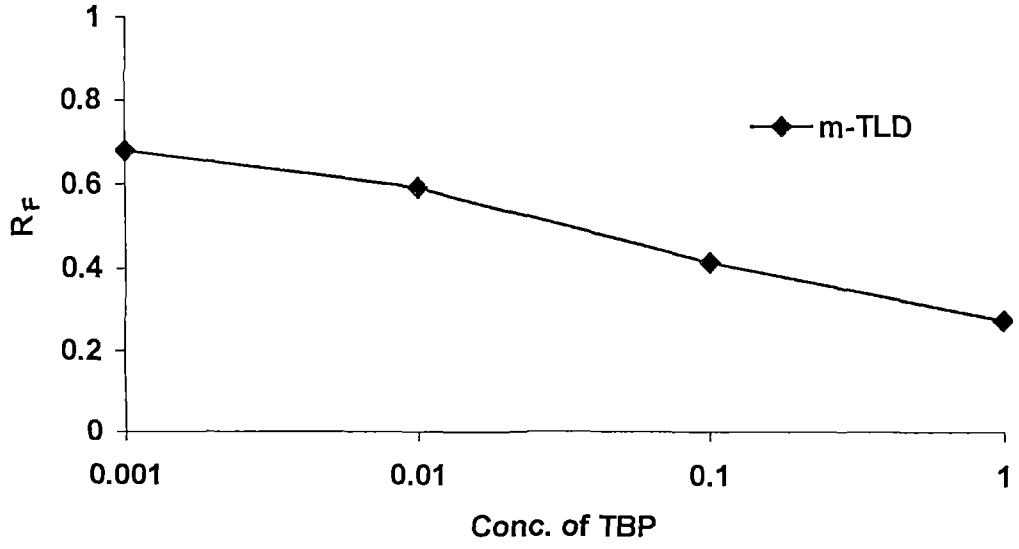

Fig.7.1(b) Plot of R_F V/c Conc. of TBP

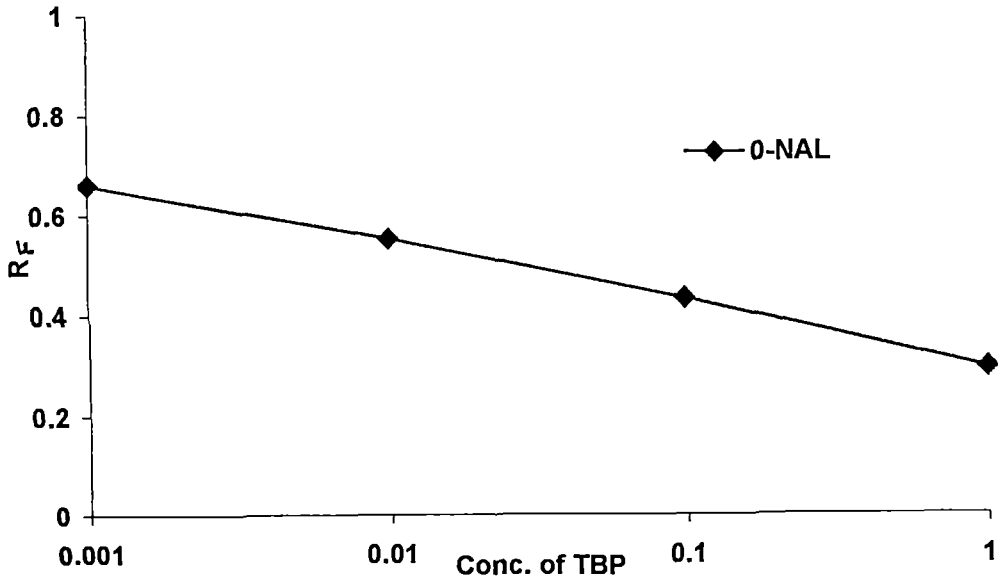

Fig.7.1(c) Plot of R_F V/s Conc. of TBP

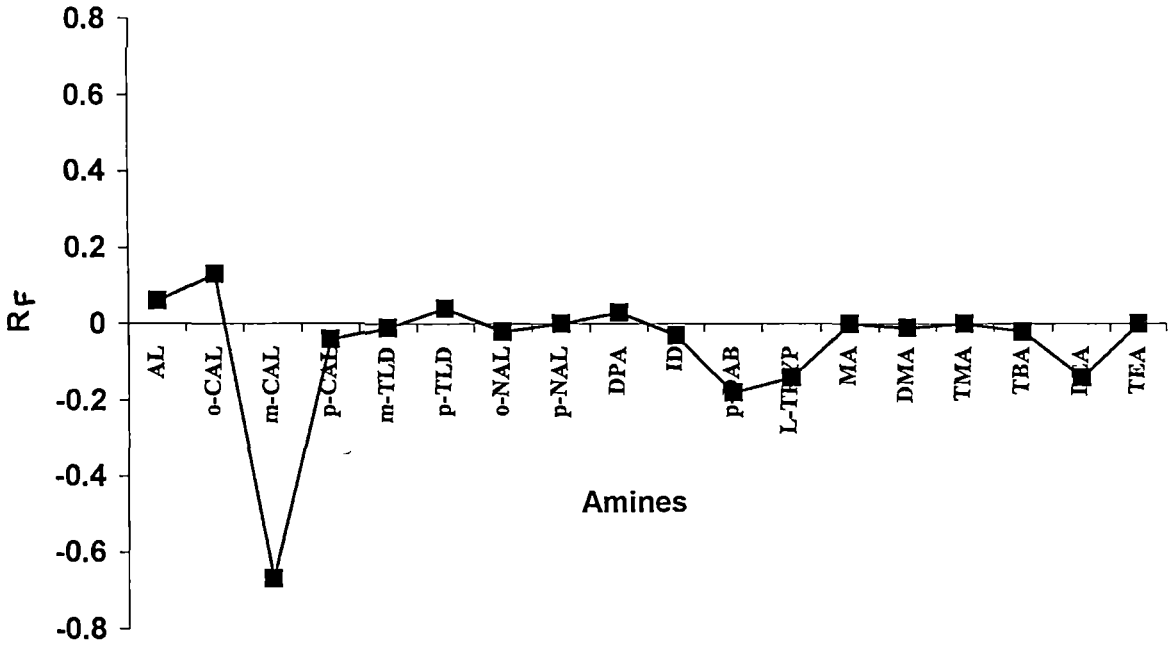

Fig.72a Plot of R_F V/s Amines

$\Delta R_F = R_F$ unimpregnated layers - R_F impregnated layers

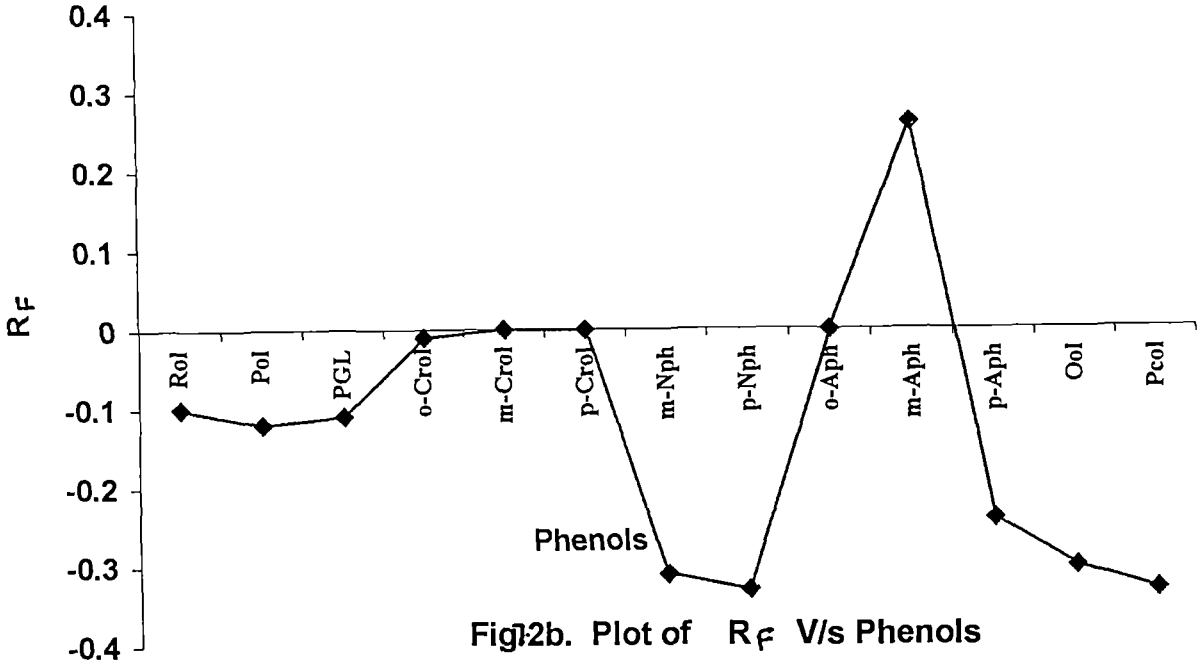

Fig.2b. Plot of R_F V/s Phenols

—◆— ΔR_F = Unimpregnated layer - R_F impregnated layer

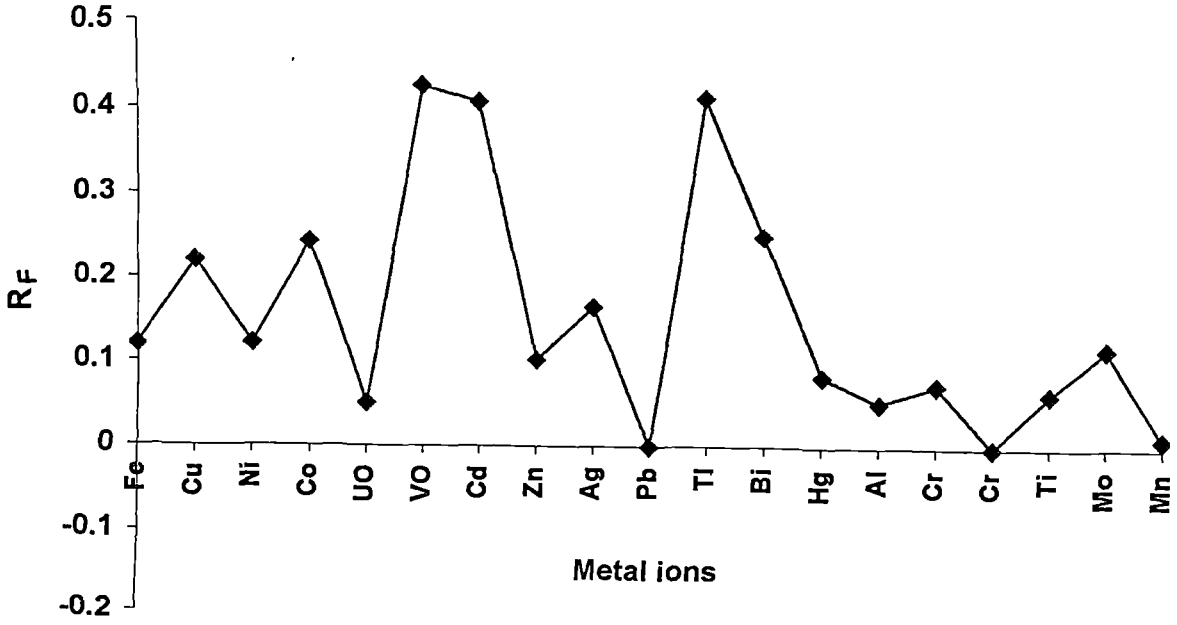

Fig.2c Plot of R_F V/s metal ions

—◆— ΔR_F = Unimpregnated layer - R_F impregnated layer

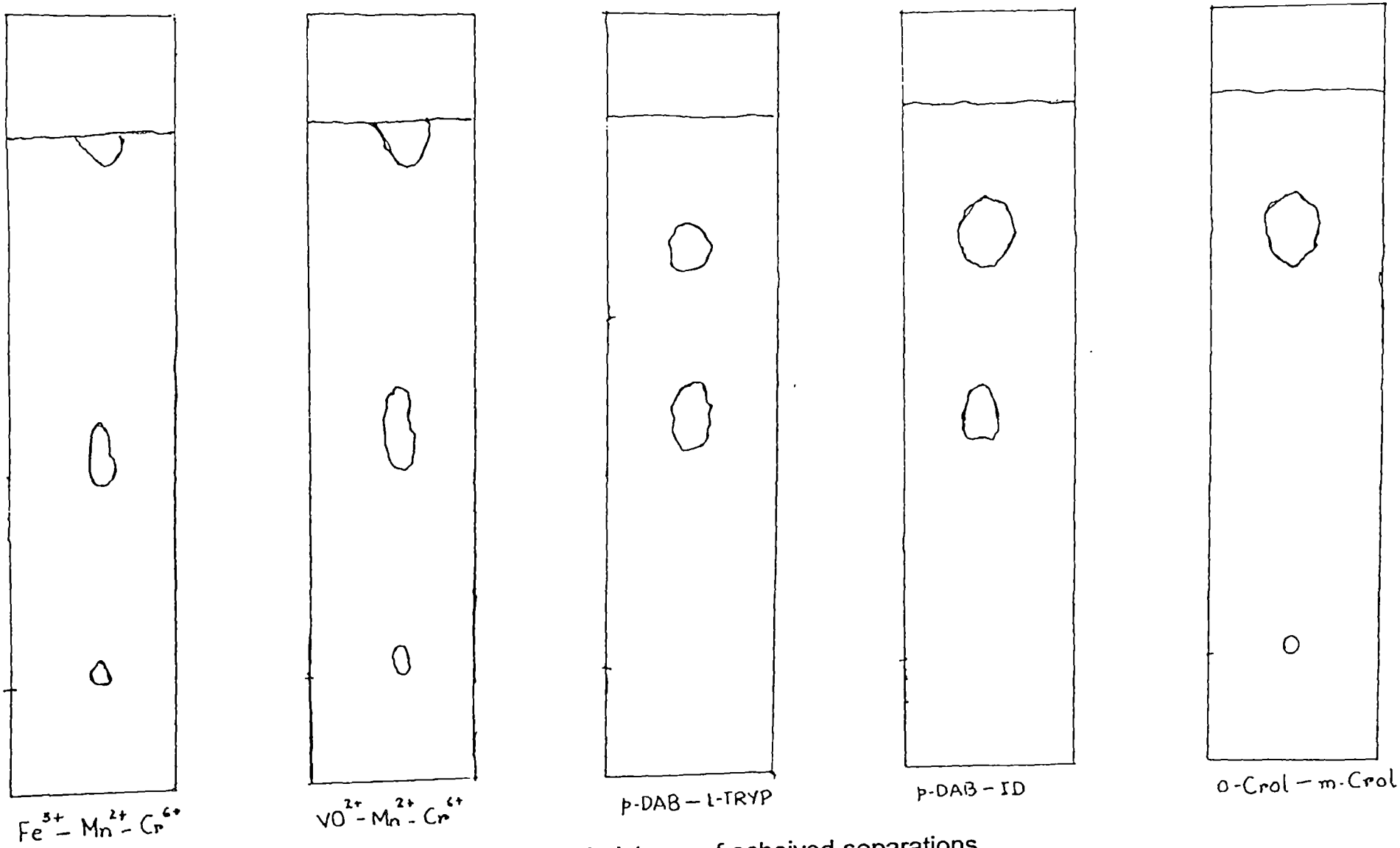

Figure 7.3 Traced pictures of acheived separations

Thin-Layer Chromatographic Studies of the Mobility of Pesticides through Soil-Containing Static Flat-Beds

Ali Mohammad*, **Iftkhar Alam Khan**, and **Nahid Jabeen**

Key Words:

Pesticides
Soil TLC
CTAB eluent
Qualitative Analysis
Mixed Layers

Summary

The chromatographic behavior of some pesticides has been studied on silica, soil, and mixed layers containing soil, with aqueous ammonium or sodium salt solutions, with or without added *N*-cetyl-*N,N,N*-trimethylammonium bromide (CTAB), with pure organic solvents, and with aqueous CTAB systems. One interesting aspect of this study is the migration of pesticides such as phosphamidon and dimethoate through pure soil. Several important binary separations have been achieved, sulfur-containing pesticides have been successfully separated from those with chlorine, phosphorus, or both phosphorus and sulfur in their molecules. Phosphorus-containing pesticides were found to migrate faster than those not containing phosphorus, in a variety of mobile phases, irrespective of the nature of the stationary phase. The trend in the relative mobilities of pesticides on the different stationary phases used is also reported.

1 Introduction

Planar chromatography is regarded as one of the simplest analytical techniques with general efficiency for the separation of organic and inorganic substances [1–6]. The convenience and cost-effectiveness of thin-layer chromatography (TLC) have resulted in its wide range of applicability in the separation and identification of pesticides and related agrochemicals [7–13]. TLC and HPTLC complement other more sensitive and precise primary methods (e.g. GLC, HPLC, SFC, and capillary electrophoresis) used for pesticide analysis. Most reported TLC methods involve the use of mixed organic solvent systems containing benzene, hexane, chloroform, acetonitrile, methanol, ethyl acetate or dichloromethane as one of the components. Of the layer materials examined, silica gel and alumina have been the most favored. A few studies have also been reported [7] on the use of layers prepared from barium or calcium sulfate, calcium carbonate or phosphate, soil, cellulose, C_{18}-bonded silica gel, and calcium sulfate mixed with silica gel to examine the mobility of pesticides. TLC investigation of pesticide metabolism in soil and plants, uptake of pesticides by plants from soil, and pesticide migration in soil [14–18] indicate that soil TLC has much to offer chemists interested in examining the uptake, translocation, and degradation of pesticides in the environment.

This study was performed with the aim of understanding the mobility of some pesticides through a static, flat layer of soil in contact with pure water, aqueous salt solutions, organic solvents and aqueous surfactant solutions. The aqueous systems selected as mobile phases are generally encountered in the soil surface and hence the results of transportation of pesticides through soil beds under the experimental conditions selected will be helpful in the formulation of a strategy for preventing the migration of harmful pesticides into the soil bed. We have also examined the mobility of pesticides through soil modified with silica gel, alumina, and cellulose to determine the effect of additives on the mobility of pesticides.

2 Experimental

2.1 Chemicals and Reagents

Silica gel G was from Merck (India) and *N*-cetyl-*N,N,N*-trimethylammonium bromide (CTAB) from CDH (India). Other reagents (cellulose, kieselguhr, ammonium sulfate, sodium chloride, methanol, toluene, cyclohexane, ethyl acetate,

A. Mohammad, I.A. Khan, and N. Jabeen, Analytical Laboratory; Department of Applied Chemistry, Faculty of Engineering, Aligarh Muslim University, Aligarh 202002, India.

benzene, etc.) were of analytical reagent grade. The pesticides studied – chloropyrifos, cypermethrin, dimethoate, endosulfan, fenvalerate, and phosphamidon (**Table 1**) – were from Bayer (India) and were used as received. All test solutions of pesticides were prepared in methanol.

2.2 Soil Samples

We used five samples (S_1–S_5) of natural, uncultivated soils collected from the soil surface horizon (0–20 cm deep) at different places in the district of Aligarh (India). The samples were dried, ground, and passed through a 100-mesh sieve to furnish a uniform particle size. The physical properties of the soil samples are given in **Table 2**.

2.3 Chromatography

Chromatography was performed on silica gel G, cellulose, kieselguhr, soil, soil + cellulose, soil + silica gel G, and soil + kieselguhr (1:1, 7:3, and 3:7). The mobile phases used are listed in **Table 3**.

2.3.1 Preparation of TLC Plates

Conventional Thin-Layer Plates

TLC plates were prepared by mixing silica gel G with double-distilled water in a 1:3 ratio. The resulting slurry was mechanically shaken for 5 min and then coated as 0.25 mm layers, on to 20 cm × 3.5 cm glass plates, by means of a Toshniwal (India) thin-layer chromatography apparatus. The plates were first dried in air at room temperature and then activated by heating at 100°C for 1 h. After activation, the plates were kept in an air-tight chamber until used. Cellulose, alumina, and kieselguhr TLC plates were prepared similarly.

Soil Thin-Layer Plates

A soil sample was slurried mechanically for 5 min after mixing with double distilled water in the ratio 1:3. The resulting homogeneous slurry was spread on to 20 cm × 3.5 cm glass plates as 0.25 mm thick layers. The plates were dried in air at room temperature (30°C) and stored in air-tight chamber until used.

Table 1

IUPAC name, chemical formula, and solubility in water of the pesticides studied.

Commercial name	Abbreviation	IUPAC name	Chemical formula
Chloropyrifos	CLPS	*O,O*-Diethyl-3,5,6-trichloro-2-pyridyl phosphorothioate	$C_9H_{11}C_{l3}NO_3PS$
Cypermethrin	CMN	(Rs)-α-Cyano-3-phenoxybenzyl-(1Rs)-*cis,trans*-3-(2,2-dichlorovinyl)--2,2-dimethylcyclopropanecarboxycate	$C_{22}H_{19}C_{l2}NO_3$
Dimethoate	DM	*O,O*-Dimethyl *s*-methyl carbamoylmethyl phosphorodithioate	$C_5H_{12}NO_3PS_2$
Endosulfan	ESN	c,c-(1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylene)dimethyl sulfite	$C_9H_2Cl_6O_3S$
Fenvalerate	FVL	(Rs)-α-Cyano-3-phenoxybenzyl-(Rs)-2-(4-chlorophenyl)-3-methyl butyrate	$C_{25}H_{22}ClNO_3$
Phosphamidon	PHM	2-Chloro-2-diethylcarbamoyl-1-methylvinyldimethyl phosphate	$C_{10}H_{19}ClNO_5P$

Data taken from *Douglas Hastley* and *Hamish Kidd* (Eds) The Agrochemicals Handbook, 2nd edn, Royal Society of Chemistry (UK) 1987.

Table 2

pH and electrical conductivity (EC) data of the different soil samples used as stationary phases.

No.	Place of collection	Texture	pH	EC (Ω^{-1})
S1	A.M.U. Fort	Sandy Loam	8.30	0.874
S2	Dhurrah Aligarh			
	(a) Sewage water-irrigated soil	Sandy Loam	8.20	0.871
	(b) Tube well-irrigated soil	Sandy Loam	7.72	0.870
S3	Tappal Soil	Clay	8.20	0.874
S4	Jattari Soil	Loam	7.89	0.867
S5	Botany Department Soil, AMU, Aligarh	Sandy Loam	7.70	0.869

Mixed Soil TLC Plates

Soil mixed with silica gel, kieselguhr, cellulose, or alumina in different ratios (50:50, 70:30, 30:70, by weight) were slurried with double distilled water in a 1:3 ratio by shaking for 5 min. This slurry was used to prepare thin layers under the same experimental conditions as described above for soil thin layer plates.

2.3.2 Procedure

The pesticide solutions (5–10 μL) were spotted on TLC plates by means of a micropipette. The spots were dried at room temperature before development. Glass jars (24 cm × 6 cm) containing the mobile phase were covered with lids and left for 10 min for saturation before introducing the plates for development. The plates were developed by the ascending technique. Solvent ascent was fixed at 10 cm. After

Table 3

The mobile phases used.

Code	Composition	Code	Composition
M1	Water	M2	0.01 m Ammonium sulfate
M3	0.05 m Ammonium sulfate	M4	0.10 m Ammonium sulfate
M5	0.5 m Ammonium sulfate	M6	1 m Ammonium sulfate
M7	1.5 m Ammonium sulfate	M8	2.0 m Ammonium sulfate
M9	0.01 m Sodium chloride	M10	0.05 m Sodium chloride
M11	0.10 m Sodium chloride	M12	0.5 m Sodium chloride
M13	1.0 m Sodium chloride	M14	1.5 m Sodium chloride
M15	2.0 m Sodium chloride	M16	1% CTAB + 0.5 m ammonium sulfate
M17	1% CTAB in water	M18	1% CTAB + 0.5 m sodium chloride
M19	Methanol	M20	Cyclohexane
M21	Benzene	M22	Hexane
M23	Ethyl acetate	M24	Toluene

development, the plates were dried and the pesticides were detected as dark brown/yellow spots by exposure to iodine vapor.

3 Results and Discussion

The results obtained have been summarized in Tables 4–8. The mobility (or R_F) data given in **Table 4** indicate that none of the pesticides migrates through the soil bed whereas PHM, CLPS, and DM are significantly mobile on silica layers developed with distilled water. Thus soil interacts with the pesticides much more strongly than to silica gel. To understand the nature of the interaction of these pesticides with different adsorbent layers, several stationary and mobile-phase systems were tested; the results obtained have been summarized in **Tables 5** and **6**. The data listed in these tables enable the following observations with regard to the mobility of pesticides through different static planar layers.

3.1 Silica layers

3.1.1 Aqueous Salt Solutions as Mobile Phases

When solutions of ammonium sulfate of different concentration were used as mobile phases pesticides such as ESN, CMN, and FVL remained at the point of application irrespective of concentration. The R_F value of PHM increased from 0.67 to 0.9 and that of CLPS decreased from 0.92 to 0.78 when the concentration of ammonium sulfate in the mobile phase was increased from 0.01 to 2 m. Peculiar behavior was observed for DM – although a single spot was obtained for high (1.5–2 m) and low (0.01–0.05 m) concentrations of ammonium sulfate, double spots were obtained with 0.1 or 1 m ammonium sulfate and triple spots with 0.5 m ammonium sulfate. This formation of multiple spots indicates the possible occurrence of different species of DM. With mobile phases containing different concentrations of sodium chloride ESN, CMN, and FVL remained at the point of application. DM furnished double spots over the entire concentration range (0.01–2 m) of sodium chloride. PHM remained more or less in the middle of the plate ($R_F \approx 0.67$–0.42) whereas the mobility of CLPS was greater ($R_F \approx 0.71$–0.95) irrespective of sodium chloride concentration.

3.1.2 Aqueous Salt Solutions with Added Surfactant as Mobile Phases

When distilled water, 0.5 m aqueous ammonium sulfate, or sodium chloride containing 1% added CTAB was used as the mobile phase, all pesticides remained at the point of application. All pesticides were clearly detected but the detection of PHM was difficult after use of 0.5 m sodium chloride containing 1% CTAB.

3.1.3 Organic Solvent Mobile Phases

With benzene as the mobile phase, no mobility was observed for any of the pesticides. With other organic solvents, however, e.g. hexane and toluene, significant mobility was observed for CMN, FVL, and ESN ($R_F \approx 0.82$, 0.87, and 0.70 respectively). This observation is important chromatographically, because these pesticides were found to stay at their point of application with all the mobile phases mentioned in Sections 3.1.1 and 3.1.2. Double spots were observed for CLPS and DM with cyclohexane and methanol, respectively. The behavior of PHM was irregular – it remained at the point of application when cyclohexane, benzene, and hexane were used as mobile phases, tailing slightly when toluene was used ($R_F \approx 0.25$), and was highly mobile when methanol and ethyl acetate were used as mobile phases ($R_F \approx 0.52$ and 0.70, respectively). CLPS migrated with most of the organic solvents ($R_F \approx 0.92$–0.94), although benzene and hexane were exceptions – with these CLPS remained very close to the origin ($R_F \approx 0.05$ and 0.18, respectively).

Table 4

Mobilities of pesticides on silica gel and soil layers developed with water (M1).

Pesticide	Silica gel (R_F)	Soil (R_F)
PHM	0.7	0.0
CLPS	0.92	0.0
ESN	0.0	0.0
CMN	0.0	0.0
FVL	0.07	0.0
DM	0.67	0.0

Table 5
Mobilities (R_F values) of pesticides on single-phase stationary phases developed with different mobile phases (M2–M24).

Stationary phase	Mobile phase	R_F value
Silica Gel	M2	PHM (0.67), CLPS (0.92), DM (0.75), (ESN = CMN = FVL = 0.0)
	M3	PHM (0.62), CLPS (0.92), DM (0.85), (ESN = CMN = FVL = 0.0)
	M4	PHM (0.58), CLPS (0.87), DM (0.50, 0.90), (ESN = CMN = FVL = 0.0)
	M5	PHM (0.57), CLPS (0.82), DM (0.82, 0.45, 0.0), (ESN = CMN = FVL = 0.0)
	M6	PHM (0.41), CLPS (0.72), DM (0.66, 0.02), (ESN = CMN = FVL = 0.0)
	M7	PHM (0.79), CLPS (0.77), DM (0.78), (ESN = CMN = FVL = 0.0)
	M8	PHM (0.90), CLPS (0.78), DM (0.66), (ESN = CMN = FVL = 0.0)
	M9	PHM (0.80), CLPS (0.90, 0.0), DM (0.87, 0.0), (ESN = CMN = FVL = 0.0)
	M10	PHM (0.54), CLPS (0.90), DM (0.82, 0.60), (ESN = CMN = FVL = 0.0)
	M11	PHM (0.67), CLPS (0.87), DM (0.60, 0.0), (ESN = CMN = FVL = 0.0)
	M12	PHM (0.62), CLPS (0.92), DM (0.85.0.0), (ESN = CMN = FVL = 0.0)
	M13	PHM (0.52), CLPS (0.71), DM (0.80.0.0), (ESN = CMN = FVL = 0.0)
	M14	PHM (0.42), CLPS (0.93), DM (0.82.0.0), (ESN = CMN = FVL = 0.0)
	M15	PHM (0.42), CLPS (0.95), DM (0.80.0.0), (ESN = CMN = FVL = 0.0)
	M16	PHM = CLPS = DM = ESN = CMN = FVL = 0.0
	M17	PHM = CLPS = DM = ESN = CMN = FVL = 0.0
	M18	PHM (ND), CLPS = DM = ESN = CMN = FVL = 0.0
	M19	PHM (0.52), CLPS (0.92), DM (0.90, 0.0), ESN = CMN = FVL = 0.0
	M20	CLPS (0.16, 0.0), PHM = DM = ESN = CMN = FVL = 0.0
	M21	PHM = CLPS = DM = ESN = CMN = FVL = 0.0
	M22	CLPS (0.18), ESN (0.70), CMN (0.25), FVL (0.25), (PHM = DM = 0.0)
	M23	PHM (0.70), CLPS (0.95), DM (0.50), (ESN = CMN = FVL = 0.0)
	M24	PHM (0.25), CLPS (0.92), CMN (0.87), FVL (0.87), (ESN = CMN = 0.0)
Soil	M10	PHM (0.95), DM (0.92, 0.0), ESN (ND) (ESN = CMN = FVL = 0.0)
	M11	PHM (ND), CLPS (0.92), DM (0.87,0.0), (ESN = CMN = FVL = 0.0)
	M12	PHM (0.95), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M13	PHM (ND), ESN(ND), (CLPS = DM = CMN = FVL = 0.0)
	M14	PHM (ND), (ESN = CLPS = DM = CMN = FVL = 0.0)
	M15	PHM = ESN = CLPS = DM = CMN = FVL = 0.0
	M2	PHM (0.92), (CLPS = ESN = CNIN = FVL = DNI = 0.0)
	M3	PHM (0.75), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M4	PHM (0.90), (CLPS = ESN = CMN = FVL = DM = 0.0)
	MS	PHM (0.87), CLPS (0.95), DNI (0.87), CNIN (0.90) (ESN = FVL = 0.0)
	M6	PHM (0.93), DM (0.92), (CLPS = ESN = CNIN = FVL = 0.0)
	M7	PHM (0.85), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M8	PHM (0.90), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M16	PHM (ND), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M17	PHM (ND), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M18	PHM (ND), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M19	PHM (ND), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M20	PHM (ND), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M21	PHM (ND), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M22	PHM (ND), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M23	PHM (ND), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M24	PHM (ND), (CLPS = ESN = CMN = FVL = DM = 0.0)
Cellulose	M5	PHM (0.90), CLPS (0.97), DNI (0.91), FVL (0.05) (ESN = CMN = 0.0)
	M12	PHM (0.95), CLPS (0.95), DNI (0.90), (ESN = CMN = FVL = 0.0)
Kieselguhr	MS	PHM (0.87), CLPS (0.87), FVL (0.05), DM (0.77, 0.0) (ESN = CMN = 0.0)
	M12	PHM (0.82), CLPS (0.62.0.90), FVL (0.10), DM (0.75) (ESN = CMN = 0.0)
Alumina	M5	PHM (0.0), (CLPS = ESN = CMN = FVL = DM = 0.0)
	M12	PHM (0.88), CLPS (0.95), DM (0.92), (ESN = CMN = FVL = 0.0)

Table 6

Mobilities (R_F values) of pesticides on biphasic stationary phases developed with different mobile phases (M1, M12, and M5).

Stationary phase	Mobile phase	R_F value
Soil + Cellulose		
1:1	M1	(PHM = ESN = ND), (CLPS = DM = FVL = CMN = 0.0)
3:7	M1	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
7:3	M1	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
1:1	M12	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
3:7	M12	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
7:3	M12	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
1:1	M5	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
3:7	M5	PHM (0.92), (ESN = CLPS = DM = FVL = CMN = 0.0)
7:3	M5	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
Soil + silica gel		
1:1	M1	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
3:7	M1	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
7:3	M1	PHM(0.85), DM (0.92, 0.0), (ESN = CLPS = FVL = CMN = 0.0)
1:1	M12	DM(0.85, 0.0), (ESN = CLPS = DM = FVL = CMN = 0.0)
3:7	M12	DM(0.80, 0.0), PHM (ND), (ESN = CLPS = FVL = CMN = 0.0
7:3	M12	PHM (0.78), DM (0.87.0.0) (ESN = CLPS = FVL = CMN = 0.0)
1:1	M5	PHM(ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
3:7	M5	DM (0.80, 0.0), PI = IM (ND), (ESN = CLPS = FVL = CMN = 0.0)
7:3	M5	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
Soil + kieselguhr		
1:1	M1	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
3:7	M1	(PHM = DM = ND), (ESN = CLPS = FVL = CMN = 0.0)
7:3	M1	(ESN = DM = ND), PHM (0.90), (CLPS = FVL = CMN = 0.0)
1:1	M12	DM(0.92, 0.0), PHM (ND), (ESN = CLPS = FVL = CMN = 0.0)
3:7	M12	DM(0.87, 0.0), PHM (ND), (ESN = CLPS = FVL = CMN = 0.0)
7:3	M12	DM (0.90), PHM (ND), (ESN = CLPS = FVL = CMN = 0.0)
1:1	M5	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
3:7	M5	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)
7:3	M5	PHM (ND), (ESN = CLPS = DM = FVL = CMN = 0.0)

On the basis of the migration behavior of pesticides on silica layers discussed several interesting conclusions can be drawn:

(i) The hydrocarbon chain length of pesticide molecules do not influence the R_F value, as is apparent from the R_F values of FVL ($C_{25}H_{22}ClNO_3$) and CMN ($C_{22}H_{19}Cl_2NO_3$), the mobility of which was identical (both remained near or at the point of application when the TLC plates were developed with water or aqueous salt solutions (M2–M15).

(ii) The presence of phosphorus in the pesticide molecule enhances the mobility. For example, PHM ($C_{10}H_{19}ClNO_5P$) was more mobile than CMN ($C_{22}H_{19}Cl_2NO_3$) or FVL ($C_{25}H_{22}ClNO_3$) when water and in aqueous salt solutions (M2–M15) were used as mobile phases. The greater mobility of PHM might be attributed to its stronger complexation with the mobile phase. The greater mobility of P-containing PHM compared with S-containing ESN (Table 4a) also supports the observation that phosphorus promotes mobility.

(iii) Pesticides with higher P/S ratios have higher mobilities as is apparent from comparison of CLPS ($C_9H_{11}C_{13}NO_3PS$) and DM ($C_5H_{12}NO_3PS_2$). The mobility of CLPS ($R_F \approx 0.92$) was greater than that of DM ($R_F \approx 0.67$) when water was used as mobile phase. With salt solutions as mobile phases (M2–M5) the mobility of CLPS was still greater ($R_F \approx 0.71$–0.95) than that of DM ($R_F \approx 0.60$–0.85). Thus TLC can be used to distinguish between pesticides containing both P and S ligands in the same molecule.

3.2 Soil Layers

3.2.1 Aqueous Salt Solution as Mobile Phases

Throughout the concentration range of sodium chloride (0.05–2 m) investigated ESN, CMN, and FVL were either not detected clearly (occasionally) or remained at the point of application (usually). PHM was more mobile ($R_F \approx 0.95$) when 0.05 or

Table 7

Trends in pesticide mobility on the different stationary phases used.

Mobile phase	Trends
CLPS	
M1	Silica gel > soil = soil + silica gel (3:7) = soil + kieselguhr (3:7) = soil + cellulose (1:7)
M5	Silica gel > soil = soil + silica gel = soil + kieselguhr = soil + cellulose
M12	Silica gel > soil = soil + kieselguhr = soil + silica gel = soil + cellulose
PHM[a)]	
M1	Soil + kieselguhr > soil + silica gel > silica gel > soil
M5	Soil > soil + cellulose > silica gel
M12	Soil > silica gel
DM	
M1	Soil + silica gel > silica gel > soil + kieselguhr = soil + cellulose = soil
M5	Soil > silica gel[b)] > soil + silica gel[c)] > soil + kieselguhr = soil + cellulose
M12	Soil + kieselguhr[c)] > silica gel[c)] > soil + silica gel[c)] > soil = soil + cellulose

[a)]PHM could not be detected on other stationary phases.
[b)]Triple spots (R_F 0.82, 0.45, and 0.0); R_F 0.82 is used for comparison purposes.
[c)]Double spots (second spot always at $R_F = 0.0$). The higher R_F value is taken for comparison. ESN, CMN and FVL remain at or near the point of application on all stationary phases.

Table 8

Separations achieved experimentally on silica gel, soil, and modified soil layers.

Stationary phase	Mobile phase	Separation (R_F)		
Silica gel	M2	DM (0.72)	from	ESN, CMN, or FVL (0.0)
		CLPS (0.92)	from	ESN, CMN, or FVL (0.0)
		PHM (0.67)	from	ESN, CMN, or FVL (0.10)
	M19	PHM (0.52)	from	ESN, CMN, or FVL (0.0)
		CLPS (0.92)	from	ESN, CMN, or FVL (0.0)
	M22	ESN (0. 70)	from	PHM, DM, CMN, or FVL (0.25)
	M9	DM (0. 82)	from	CMN or FVL (0.0)
		CLPS (0.90)	from	ESN, CMN, or FVL (0.10)
		PHM (0.75)	from	ESN, CMN, or FVL (0.0)
Soil	M2	DM (0.90)	from	CMN, CLPS, ESN, or FVL (0.0)
		PHM (0.90)	from	CMN, CLPS, ESN, or FVL (0.0)
	M9	DM (0.85)	from	CMN, CLPS, ESN, or FVL (0.0)
		PHM (0.87)	from	CMN, CLPS, ESN, or FVL (0.0)
Soil + silica gel (7:3)	M1	PHM (0.85)	from	CLPS, ESN, CMN, or FVL (0.0)
Soil + silica gel (7:3)	M12	PHM (0.78)	from	CLPS, ESN, CMN, or FVL (0.0)
Soil + kieselguhr (7:3)	M1	PHM (0.90)	from	CLPS, CMN, or FVL (0.0)
Soil + cellulose (1:7)	M5	PHM (0.92)	from	CLPS, ESN, CMN, FVI, or DM (0.0)

0.5 m sodium chloride was used as mobile phase. At higher sodium chloride concentrations, it could not be detected. Double spots were obtained for DM when plates were developed with 0.05 m ($R_F \approx 0.0$ and 0.92) or 0.1 m ($R_F \approx 0.0$ and 0.87) aqueous sodium chloride. CLPS was mobile only when 0.10 m sodium chloride was used as mobile phase ($R_F \approx 0.92$).

With different concentrations of ammonium sulfate (0.01–2 m) as mobile phase PHM was highly mobile at all concentrations (RF ≈ 0.75–0.93). CLPS ($R_F \approx 0.95$) and CMN ($R_F \approx 0.90$) were also more mobile when chromatographed with 1.0 m aqueous ammonium sulfate solution as mobile phase. DM moved with the solvent front when aqueous ammonium sulfate (0.05–1.0 m) solutions were used as mobile phase. All other pesticides remained at the origin, irrespective of the concentration of ammonium salt in the mobile phase.

3.2.2 Aqueous Salt Solutions with Added Surfactant as Mobile Phases

When 0.5 m aqueous sodium or ammonium salt solutions containing 1% CTAB (M16 and M18) were used as mobile phase all pesticides remained at the point of application. PHM could not be detected clearly after use of M18.

3.2.3 Organic Solvent Mobile Phases

All the pesticides remained at the point of application irrespective of the nature of the organic mobile phase (M19–M24). PHM was not detected.

3.3 Mixed Layers Containing Soil

The results obtained on various mixed layers are briefly discussed below.

3.3.1 Soil Mixed with Cellulose

When soil mixed with cellulose (1:1, 7:3, 3:7) was used as the stationary phase in combination with water as mobile phase all the pesticides except PHM and ESN were well detected, and remained at the point of application. With 0.5 m sodium chloride as mobile phase, all the pesticides stayed at the point of application and PHM could not be detected. Interestingly, PHM was very mobile ($R_F \approx 0.92$) when 0.5 m ammonium sulfate was used as mobile phase with soil + cellulose, 3:7, as stationary phase.

3.3.2 Soil Mixed with Silica Gel

PHM was very mobile on layers prepared from soil + silica, 7:3, with water ($R_F \approx 0.8$) and 0.5 m sodium chloride ($R_F \approx 0.78$) as mobile phases. Its detection on other mixed layers developed with water or aqueous salt solutions was difficult. Double spots were observed for DM with some TLC systems (water as mobile phase, soil + silica gel, 7:3; 0.5 m sodium chloride as mobile phase, soil + silica gel, 7:3, 3:7, and 1:1; and 0.5 m ammonium sulfate as mobile phase, soil + silica gel, 3:7). ESN, CLPS, FVL, and CMN always remained at the point of application.

3.3.3 Soil Mixed with Kieselguhr

ESN and DM could not be detected on soil + kieselguhr, 1:1, 7:3, or 3:7. With water as mobile phase, PHM migrated with the mobile phase front ($R_F \approx 0.90$) on the soil + kieselguhr, 7:3. Other pesticides remained at the point of application. When 0.5 m sodium chloride was used as mobile phase, all but DM remained at the point of application. Double spots were for DM on layers of 1:1 ($R_F \approx 0.92$, 0.0) and 3:7 ($R_F \approx 0.87$, 0.0) soil + kieselguhr, whereas a single spot ($R_F \approx 0.90$) was obtained on the 7:3 layer. None of the pesticides was mobile when 0.5 m ammonium sulfate was used as mobile phase.

3.4 Miscellaneous Layers

To compare the retention efficiency of cellulose, kieselguhr, and alumina as layer materials, pesticides were chromatographed with 0.5 m sodium chloride and ammonium sulfate solutions as mobile phases. The results obtained are summarized in Table 5. The retention patterns observed were:

(i) With 0.5 m sodium chloride, the mobility of PHM increases in the order silica gel < kieselguhr < alumina < cellulose. CLPS moved with the mobile phase front ($R_F > 0.9$) irrespective of the nature of the stationary phase. An additional spot at the point of application was also observed for CLPS on the kieselguhr layer. The R_F value of DM was in the order kieselguhr < silica gel < cellulose < alumina; two spots were obtained on the silica layer. The R_F of the second spot was 0.0.

(ii) With 0.5 m ammonium sulfate as mobile phase all the pesticides remained at the origin ($R_F \approx 0$) on the alumina layer. The mobility trends for PHM and CLPS were in the order alumina < silica gel < kieselguhr < cellulose. Triple spots were obtained for DM on silica gel; double spots on kieselguhr, and a single spot on cellulose.

(iii) With 0.5 m sodium chloride and ammonium sulfate as mobile phases, FVL, ESN, and CMN always remained at the point of application.

These results indicate that the multiplicity of pesticides spots depends upon the nature of stationary phase.

Table 7 summarizes the mobility trends for CLPS, PHM, and DM on different single and biphasic layers developed with water, and with aqueous solutions (0.5 m) of sodium chloride or ammonium sulfate. From this table it is clear that the mobility of CLPS is greater on silica than on soil or mixed layers, irrespective of mobile phase (M1, M5, M12). PHM and DM migrated more quickly through soil or mixed-soil layers.

The separations obtained experimentally with different TLC systems are listed in Table 8. These separations are especially important because it is possible to separate S-containing pesticides from those containing Cl, P, or both P and S. Similarly, mixtures of DM and CLPS, both of which contain both S and P in their molecule, can also be separated.

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TLC studies and separation of heavy metal cations on soil amended silica gel layers developed with surfactant-mediated solvent systems

Ali Mohammad* & Nahed Jabeen

Analytical Research Laboratory, Department of Applied Chemistry, Z H College of Engineering & Technology, AMU, Aligarh 202 002, India

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Thin-layer chromatography (TLC) of ten heavy metal cations was performed on soil, silica gel and soil mixed with silica gel sorbent phases using aqueous solutions of cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulphate (SDS), polyoxyethylene dodecyl ether (Brij-35) and *iso*-octylphenoxypolyethoxy ethanol (TX-100) surfactants with or without added urea, nitrate or chloride of ammonium and sodium phosphate as mobile phases. In all, twenty-nine stationary and twenty-five mobile phases were used in order to examine the mobility pattern and to find out the best TLC system for metal cations separation from their multicomponent mixtures on soil mixed with silica gel layers. The mobility of all cations was insignificant on pure soil layers irrespective of the nature of mobile phase used. Addition of silica gel into soil bed leads to the increase of mobility and facilitates the separation of metal cations. Amongst surfactant solutions, CTAB at concentration level of 0.5 M was found to be most effective, the analytical potentiality of which was further improved in the presence of urea. The TLC system comprising of silica gel plus 0.6 M urea (1:1, *v/v*) as mobile phase was found most favourable for achieving separations of metal ions from their multicomponent mixtures. A few such separations worth mentioning include, Fe^{3+}-Cu^{2+}-Ni^{2+}-Hg^{2+}, Zn^{2+}-Cd^{2+}-Hg^{2+}, Ni^{2+}-Cu^{2+}-Fe^{3+}, Zn^{2+}-Fe^{3+}-Ni^{2+}-Hg^{2+}, Pb^{2+}-Cd^{2+}-Hg^{2+} and Ni^{2+}-Cu^{2+}-Pb^{2+}. R_F values of metal ions on soil amended with alumina, kieselguhr, cellulose and fly ash layers have also been determined. Salting – in effect exhibited by certain metal ions like Cd^{2+}, Ni^{2+} or Co^{2+}, Ag^{+}, Hg^{2+} has been reported. Effect on mobility of metal ions, by replacing urea with different fertilizers in the CTAB containing mobile phase has also been examined.

Water pollution due to heavy metal pollutants cause direct toxicity, both to humans and other living beings, due to their presence beyond specified limits. Thin layer chromatography (TLC) has made a major contribution to the analysis of inorganic cations and being low cost technique it is still enjoying the popularity as an undisputed analytical tool. The recent applications of TLC have been well-documented in reviews[1-4], books[5-7] and research papers[8-12].

A new concept for qualitative analysis by soil TLC was developed by Helling and Turner in 1968[13]. Their method was utilized by several workers to examine the mobility pattern of pesticides[14-17] and heavy metals[18-23] in various types of soils. The mobility or leachability of such chemicals through the soil bed has tremendous influence on the life process of plants. An interesting study on mobility of cadmium on twenty-two soil layers of different nature has been reported by Sanchez Camazano and Sanchez-Martin[24]. Their results clearly demonstrate the significant influence of soil properties on the mobility of cadmium.

*For correspondence (E-mail: mahammadali4u@rediffmail.com)

TLC methods reported so far for investigating the mobility of heavy metals and trace elements in soil did not consider the following two aspects:

(i) Use of surfactants as mobile phase.
(ii) Effect of presence of silica, alumina; cellulose and kieselguhr in soil bed on the mobility of heavy metal cations.

In view, of the above facts, it was considered worthwhile to examine the mobility of heavy metal cations through static planar soil bed amended with silica, alumina, cellulose and kieselguhr using aqueous solutions of cationic, anionic and non-ionic surfactants as mobile phase. The results of this study may be of immense utility to understand the role of surfactant mediated mobile phases on the mobility or leachability of heavy metals through pure and soil amended layers.

Experimental Procedure

Apparatus

A TLC apparatus (Toshniwal, India) was used to prepare silica gel layers (0.25 mm) on 20×3 cm glass plates and 24×6 cm glass jars were used.

Chemicals and reagents

Silica gel G (Merck, India), N-cetyl-N, N, N-trimethyl ammonium bromide (CTAB) of CDH India, urea (GSC, India), sodium dodecyl sulphate (SDS) of BDH India and polyoxyethylene dodecyl ether (Brij 35) and iso-octylphenoxypolyethoxy-ethanol (TX-100) of Loba Chemie, India were used.

Metal ions studied

Pb^{2+}, Bi^{3+}, Zn^{2+}, Ag^{+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Hg^{2+}, Cd^{2+} and Fe^{3+}

Test solution

Chromatography was performed on 1% standard solutions of the chloride, nitrate or sulphate salts of the above mentioned metal ions.

Soil sample

Samples (S_1-S_8) of natural uncultivated soils (Table 1) collected from the soil surface horizon (0-20 cm deep) at different locations in the district of Aligarh (India) were used. The samples were dried, grounded and passed through the 100 mesh size sieve to get uniform particle size.

Detection

Fe^{3+}, Cu^{2+} were detected with 1% potassium ferrocyanide; Ni^{2+} and Co^{2+} were detected with 1% solution of alcohlic dimethylglyoxime and Zn^{2+}, Cd^{2+} Pb^{2+}, Bi^{3+}, Ag^{+} and Hg^{2+} were detected with a solution of 0.5% dithizone in carbontetrachloride.

Mixed mobile phases were prepared by mixing different volumes of individual solution/solvent (Table 2). Stationary phases are summarized in Table 3.

Preparation of silica gel TLC plates

Plain thin-layer plates—TLC plates were prepared by mixing silica gel G with double distilled water in a 1:3 ratio. The resultant slurry was mechanically shaken for 5 min and then it was coated onto glass plates with the help of a TLC applicator to give a layer of 0.25 mm thickness. The plates were first air dried at room temperature and then activated by heating at 100°C for 1 h. After activation, the plates were kept in air tight chamber until used.

Soil thin-layer plates—To prepare soil TLC plates, soil sample was slurried mechanically by shaking for 5 min after mixing with double distilled water in a 1:3 ratio. The resultant homogeneous slurry was spread onto 20×3.5 cm glass plates as 0.25 mm thick layer. The plates were air dried at room temperature (30°C) and stored in air tight chamber until used.

Table 1—Physico-chemical properties of different soil samples

S.no	Place of collection	Physical parameter			Chemical parameter							
		Texture	*pH*	Electrical conductivity	Cation exchange capacity	Carbon (%)	organic matter (%)	K	Na	Ca	N	P
S_2	AMU fort Dhurrah,Aligarh	Sandy loam	8.30	0.874	3.6	0.56	0.97	30	15	1	0.29	28
(S_3,S_4)	i) Sewage water irrigated soil (15,20 cm)	Sandy loam	8.20	0.871	4	0.37	0.65	2	13	3	0.28	30
(S_5,S_6)	ii) Tubewell irrigated soil (15,20 cm)	Sandy loam	7.72	0.970	3.4	0.42	0.72	3	13	4	0.26	28
S_8	Tappal soil	Clay	8.20	0.874	3.7	0.89	1.54	8	47	20	0.36	40
S_7	Jattari soil	Loam	7.89	0.867	4	0.42	0.72	5	34	2	0.28	30
S_1	Botany deptt. soil	Sandy loam	7.70	0.869	6.9	0.63	1.87	39	16	1	0.26	29

Table 2—The solvent systems used as mobile phase

Solvent System	Symbol	Composition
Aqueous surfactant solution	M_1	0.1 M CTAB
	M_2	0.1 M SDS
	M_3	0.1 M TX-100
	M_4	0.1 M Brij-35
	M_5	0.01 M CTAB
	M_6	0.001 M CTAB
	M_7	0.0001 M CTAB
	M_8	0.3 M CTAB
	M_9	0.4 M CTAB
	M_{10}	0.5 M CTAB
Fertilizer added surfactant solution	M_{11}	0.5 M CTAB + 0.1 M Urea (1:1)
	M_{12}	0.5 M CTAB + 0.1 M Urea (1:9)
	M_{13}	0.5 M CTAB + 0.1 M Urea (9:1)
	M_{14}	0.5 M CTAB + 0.6 M Urea (1:9)
	M_{15}	0.5 M CTAB + 0.6 M Urea (1:1)
	M_{16}	0.5 M CTAB + 0.6 M Urea (9:1)
	M_{17}	0.5 M CTAB + 0.8 M Urea (1:1)
	M_{18}	0.5 M CTAB + 0.8 M Urea (1:9)
	M_{19}	0.5 M CTAB + 0.8 M Urea (9:1)
	M_{20}	0.5 M CTAB + 2 M Urea (1:1)
	M_{21}	0.5 M CTAB + 2 M Urea (9:1)
	M_{22}	0.5 M CTAB + 2 M Urea (1:9)
	M_{23}	0.5 M CTAB+0.6 M ammonium chloride (1:1)
	M_{24}	0.5 M CTAB+0.6 M ammonium nitrate (1:1)
	M_{25}	0.5 M CTAB+0.6 M sodium phosphate (1:1)

Mixed soil TLC plates—Silica gel, kieselguhr, cellulose or alumina were mixed with soil in 9:1 ratio by weight. The contents were slurried with double distilled water in a 1:3 ratio by shaking for 5 min. Using this slurry, thin layers were prepared under the same experimental conditions as described above for soil layer plates.

Method

Chromatography—The metal ion solutions (5-10 μL) were spotted on TLC plates with micropipette. The spots were dried at room temperature before development. The glass jars containing mobile phase were covered with lids and left for 10 min for saturation before introducing the plates for development. The plates were developed with chosen solvent system by ascending technique. The solvent ascent was fixed at 10 cm in all cases. After development, the plates were dried and the spots of metal ions were detected using appropriate reagent.

The reproducibility of R_F values on S_{11} developed with M_{15} was checked by three independent analyses and by the same analyst on different days under identical experimental conditions, in the same laboratory, using the same apparatus.

Table 3—Stationary phase

Symbol	Composition
(A)	Pure silica gel
(B)	Pure soil
S_1 -	Soil botany Deptt.
S_2 -	Soil A.M.U. Fort
S_3 -	Dhurrah sewage irrigated Soil (15 cm depth)
S_4 -	Dhurrah sewage irrigated Soil (20 cm depth)
S_5 -	Dhurrah tubewell irrigated Soil (15 cm depth)
S_6 -	Dhurrah tubewell irrigated Soil (20 cm)
S_7 -	Jattari soil
S_8 -	Tappal soil
(C)	
S_9 -	Silica gel mixed with soil type S_1 (9:1)
S_{10} -	Silica gel mixed with soil type S_2 (9:1)
S_{11} -	Silica gel mixed with soil type S_3 (9:1)
S_{12} -	Silica gel mixed with soil type S_4 (9:1)
S_{13} -	Silica gel mixed with soil type S_5 (9:1)
S_{14} -	Silica gel mixed with soil type S_6 (9:1)
S_{15} -	Silica gel mixed with soil type S_7 (9:1)
S_{16} -	Silica gel mixed with soil type S_8 (9:1)
(D) -	Silica gel mixed with soil type S_3 in the ratios (8:2), (7:3), (6:4), (5:5), (4:6), (3:7), (2:8), (1:9)
(E)	Alumina + soil type (S_3) 9:1 Cellulose + soil type (S_3) 9 :1 Kieselguhr + soil type (S_3) 9:1, flyash + soil type (S_3) 9:1

Results and Discussion

The results presented in Table 4 reveal that the mobility of metal cations is decreased with the increase of soil concentration in the stationary phase. The soil concentration above 50% in the stationary phase was undesirable because of (a) lack of differential migration as all metal ions are strongly retained by the stationary phase near the point of application, (b) formation of tailed spots in certain cases, and (c) poor detection of Cu^{2+} and Co^{2+}.

The best results, with reasonably good detection clarity, spot compactness and differential migration of metal cations were realized on layers prepared from a mixture consisting of silica gel and soil type S_3 in 90:10 ratio (termed as S_{11} in this paper). Therefore, it was selected for detailed study. It is also evident from the results recorded in Table 4 that amongst the surfactants used, 0.1 M aqueous CTAB (M_1) is a useful eluent to achieve important separations of metal ions. With this system, Hg^{2+} showed maximum R_F value (R_F ~ 0.96) and Cd^{2+} moved nearly upto the middle of the chromatoplate ($R_F \cong 0.41$). Both the metal ions remained more or less at the point of

Table 4—R_F value (mobility trend)[a] of heavy metal cations on pure silica gel and soil (type S_3) plus silica gel mixed layers developed with aqueous solutions of different type of surfactants

Metal ion	M_1				M_2				M_3				M_4			
	Pure SG	SG+Soil 90:10	SG+ Soi 70:30	SG+ Soil 50:50	Pure SG	SG+Soil 90:10	SG+Soil 70:30	SG+Soil 50:50	Pure SG	SG+Soil 90:10	SG+Soil 70:30	SG+ Soil 50:50	Pure SG	SG+Soil 90:10	SG+Soil 70:30	SG+ Soil 50:50
Fe^{3+}	0.05	0.09	0.04	0.04	0.05	0.09	0.04	0.04	0.05	0.09	0.04	0.04	0.05	0.09	0.04	0.04
Co^{2+}	0.46	0.56	0.30	ND	0.73	0.53	0.37	ND	0.35	0.57	0.21	ND	0.40	0.56	0.24	ND
Ni^{2+}	0.60	0.56	0.35	0.17	0.90	0.52	0.46	0.21	0.45	0.56	0.22	0.13	0.43	0.55	0.27	0.12
Cu^{2+}	0.08	0.10	0.03	0.02	0.05	0.05	0.03	0.10	0.02	0.06	0.01	0.02	0.04	0.05	0.02	0.01
Zn^{2+}	0.10	0.14	0.09	0.05	0.08	0.18	0.06	0.06	0.05	0.23	0.05	0.04	0.06	0.11	0.05	0.03
Ag^{+}	0.00	0.00	0.00	0.00	0.08	0.14	0.07	0.05	0.23	0.18	0.13	0.11	0.28	0.24	0.18	0.08
Cd^{2+}	0.40	0.41	0.32	0.22	0.42	0.40	0.22	0.15	0.17	0.20	0.15	0.08	0.21	0.26	0.12	0.08
Hg^{2+}	0.97	0.96	0.96	0.97	0.35	0.27	0.13	0.10	0.17	0.22	0.09	0.05	0.12	0.21	0.07	0.05
Pb^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bi^{3+}	0.16	0.26	0.12	0.11	0.12	0.15	0.12	0.25	0.20	0.20	0.14	0.12	0.17	0.16	0.15	0.15

a) Mobility of all metal ions on pure soil layer was minimal ($R_F \cong 0.05$).
ND refers to not detected

application when 0.1 M aqueous solutions of non-ionic surfactants (M_3 & M_4) were used as the mobile phase. Similarly, Pb^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Bi^{3+} and Ag^{+} showed little mobility in aqueous surfactant solutions irrespective of the nature of the hydrophilic group of the surfactant (M_1-M_4).

It is important to mention here that none of the metal ions tends to exhibit mobility in the absence of surfactant as all the metal ions were found to remain at the point of application ($R_F \cong 0.0$) when chromatographed on soil layer (S_3) using distilled water (zero surfactant) as mobile phase.

The results of TLC of metal ions performed on selected stationary phase (S_{11}) using different CTAB concentrations (M_5-M_{10}) as mobile phase are listed in Table 5. The results reported in Table 5 indicate that the mobility of Zn^{2+}, Cd^{2+} and Hg^{2+} increased with the increase in the concentration of CTAB from 0.001 to 0.5 M. Interestingly, the clarity of spot detection was also found to be associated with the degree of concentration of CTAB. The heavy metal cations were detected with enhanced clarity when 0.5 M CTAB was used as eluent. The increased mobility of certain cations facilitates the separation of Cd^{2+} from Zn^{2+} and Hg^{2+}. Preparation of CTAB solutions with higher concentrations exceeding to 0.5 M was difficult because of solubility limitations and hence no further studies with higher concentration of CTAB could be performed. Fe^{3+}, Cu^{2+}, Ag^{+}, Pb^{2+} and Bi^{3+} remained at or near the point of application at all concentration levels. Ni^{2+} and Co^{2+} showed moderate mobility ($R_F \cong 0.49$-0.62) and gave almost identical results over the entire CTAB concentration range.

Different concentrations of urea (M_{11}-M_{22}) were added in 0.5 M CTAB in variable ratios and the results of mobility of metal ions with urea added surfactant mobile phases using S_{11} layer are tabulated in Table 6. Amongst urea added mobile phases, the mobile phase consisting of 0.5 M CTAB plus 0.6 M urea in 1:1 ratio (M_{15}) was found most useful. At this concentration level, Cu^{2+} showed slight mobility ($R_F \cong 0.38$) to facilitate its separation from Ni^{2+}, Pb^{2+}, Hg^{2+} and Fe^{3+}. It was found that when 0.1 M urea solution was added into 0.5 M CTAB in different volume ratio (M_{11}-M_{13}), Cu^{2+} remained near the point of application ($R_F \cong 0.07$-0.18). Similarly, with mobile phases such as M_{14}, M_{18} and M_{22} Cu^{2+} was strongly retained by the stationary phase giving R_F value 0.10, 0.12 and 0.08 respectively. At all concentration levels of urea in the mobile phase, metal ions such as Fe^{3+}, Zn^{2+}, Pb^{2+}, Bi^{3+} remained at the point of application or

Table 5 —R_F value of metal ions on S_{11} layer developed with different concentrations of CTAB

Metal ion	M_5	M_6	M_7	M_8	M_9	M_{10}
Fe^{3+}	0.05	0.06	0.05	0.05	0.06	0.07
Co^{2+}	0.49	0.51	0.59	0.58	0.62	0.55
Ni^{2+}	0.52	0.52	0.57	0.56	0.60	0.57
Cu^{2+}	0.06	0.06	0.06	0.06	0.05	0.07
Zn^{2+}	0.12	0.10	0.13	0.16	0.15	0.23
Ag^{+}	0.12	0.40	0.37	0.15	0.14	0.13
Cd^{2+}	0.24	0.25	0.22	0.17	0.57	0.64
Hg^{2+}	0.87	0.29	0.00	0.78	0.80	0.81
Pb^{2+}	0.00	0.00	0.00	0.00	0.00	0.00
Bi^{3+}	0.16	0.20	0.22	0.15	0.15	0.20

Table 6—R_F Value of metal ions on S_{11} developed with 0.5 M aqueous CTAB containing different concentrations of added urea

Metal ions	M_{11}	M_{12}	M_{13}	M_{14}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{20}	M_{21}	M_{22}
Fe^{3+}	0.07	0.06	0.06	0.08	0.08	0.07	0.08	0.10	0.06	0.06	0.05	0.07
Co^{2+}	0.59	0.56	0.59	0.70	0.75	0.63	0.76	0.70	0.62	0.63	0.64	0.66
Ni^{2+}	0.64	0.61	0.57	0.70	0.70	0.67	0.69	0.68	0.67	0.65	0.68	0.70
Cu^{2+}	0.18	0.07	0.07	0.10	0.38	0.38	0.31	0.12	0.30	0.35	0.33	0.08
Zn^{2+}	0.19	0.10	0.17	0.11	0.22	0.19	0.13	0.12	0.16	0.15	0.19	0.10
Ag^{+}	0.11	0.06	0.17	0.06	0.13	0.15	0.15	0.10	0.20	0.12	0.12	0.60
Cd^{2+}	0.17	0.51	0.61	0.55	0.74	0.62	0.64	0.60	0.70	0.67	0.65	0.52
Hg^{2+}	0.13	0.87	0.96	0.92	0.96	0.87	0.96	0.96	0.93	0.97	0.92	0.96
Pb^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bi^{3+}	0.17	0.22	0.15	0.19	0.24	0.20	0.20	0.22	0.17	0.22	0.17	0.21

showed very little mobility whereas Ni^{2+}, Co^{2+}, Cd^{2+} and Hg^{2+} showed considerable mobility. As better separation possibilities were dictated by M_{15} it was selected for detailed study. At first instance, the mobility behaviour of metal cations was investigated on TLC plates coated with pure different types of soil (S_1-S_8) using M_{15} as mobile phase. None of the cations show any mobility on pure soil layers and remained at the point of application (R_F = 0.05). The high *p*H value of the soil samples (*p*H > 7.0) seems to be responsible for the low mobility of cations. These results are in consonance with the observations of Sanchez[24], who have reported low mobility (R_F = 0.14) of Cd^{2+} in soils whose *p*H value is ≥ 6.3. Most of the metal ions were either not detected or remained at or near the point of application. Hg^{2+} was the exception, which migrates on S_3 and S_4 layers showing R_F value of 0.62. However, Hg^{2+} could not be clearly detected on layers prepared from S_1, S_2 and S_5-S_8.

In order to examine the effect of inorganic fertilizers on the mobility of metal ions, urea (organic fertilizer) was replaced by three other fertilizers (ammonium chloride, ammonium nitrate and sodium phosphate) in aqueous CTAB mobile phase and TLC of metal ions was performed using different soil types (S_1-S_8) mixed with silica gel in 1:9 ratio as the stationary phase (S_9-S_{16}). The results summarized in Table 7 show the following trends.

(i) Detection of metal ions was not sharp when sodium phosphate was used in the mobile phase whereas such a problem was not observed with mobile phases containing ammonium nitrate or chloride. In these cases detection of metal ions was sharp and spots were highly compact.

(ii) Co^{2+}, Ni^{2+}, Hg^{2+} and Bi^{3+} gave comparable results (i.e. almost identical mobility) irrespective of the type of fertilizer present in the mobile phase. Bi^{3+} remained near the point of application or showed very little mobility while Ni^{2+}; Co^{2+} and Hg^{2+} moved with the solvent front (M_{15}, M_{23}, M_{24}, M_{25}) on layers prepared from S_9-S_{16}.

(iii) With sodium phosphate added aqueous CTAB mobile phase (M_{25}), Cu^{2+} showed stronger interaction with the stationary phase giving the R_F value of 0.03 irrespective of the type of soil used in the stationary phase. However, an enhanced

Table 7—R_F value of metal ions obtained on TLC plates prepared of silica gel mixed with different types of soils in 9:1 ratio (S_9-S_{16}) developed with 0.5 M CTAB with added fertilizer.

Mobile Phase	Metal Ions	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}
M_{15}		0.55	0.52	0.75	0.72	0.42	0.68	0.70	0.60
M_{24}	Co^{2+}	0.78	0.76	0.66	0.76	0.81	0.75	0.85	0.64
M_{25}		0.73	0.87	0.73	0.82	0.79	0.84	0.68	0.71
M_{23}		0.87	0.79	0.82	0.90	0.84	0.51	0.89	0.82
M_{15}		0.64	0.54	0.74	0.67	0.49	0.66	0.78	0.50
M_{24}	Ni^{2+}	0.78	0.77	0.70	0.77	0.77	0.74	0.87	0.65
M_{25}		0.79	0.87	0.76	0.87	0.83	0.85	0.70	0.73
M_{23}		0.86	0.82	0.85	0.89	0.89	0.50	0.89	0.85
M_{15}		0.31	ND	0.38	0.34	0.20	0.31	0.34	ND
M_{24}	Cu^{2+}	0.26	0.19	0.19	0.23	0.23	0.26	0.24	0.21
M_{25}		0.03	0.03	0.03	0.02	0.02	0.02	0.03	0.02
M_{23}		0.33	0.30	0.23	0.37	0.35	0.25	0.33	0.27
M_{15}		0.12	0.10	0.22	0.23	0.05	0.14	0.17	0.11
M_{24}	Zn^{2+}	0.17	0.12	0.14	0.12	0.16	0.14	0.13	0.14
M_{25}		0.57	0.55	0.30	0.52	0.41	0.45	0.60	0.44
M_{23}		0.14	0.13	0.17	0.17	0.28	0.12	0.25	0.10
M_{15}		0.65	0.50	0.74	0.72	0.36	0.65	0.63	0.47
M_{24}		0.76	0.78	0.71	0.75	0.77	0.82	0.76	0.70
M_{25}	Cd^{2+}	0.72, 0.22	0.77, 0.20	0.49T	0.74, 0.18	0.69, 0.16	0.77, 0.14	0.74, 0.20	0.69
M_{23}		0.75	0.72	0.72	0.85	0.81	0.40	0.82	0.78
M_{15}		0.92	0.93	0.96	0.99	0.80	0.97	0.93	0.87
M_{24}	Hg^{2+}	0.92	0.93	0.87	0.87	0.88	0.96	0.93	0.92
M_{25}		0.88	0.81	0.96	0.87	0.96	0.92	0.96	0.89
M_{23}		0.93	0.80	0.91	0.97	0.88	0.57	0.90	0.95
M_{15}		0.21	0.17	0.24	0.18	0.17	0.22	0.23	0.15
M_{24}	Bi^{3+}	0.19	0.14	0.15	0.25	0.36	0.23	0.22	0.14
M_{25}		0.12	0.10	0.15	0.16	0.14	0.16	0.12	0.17
M_{23}		0.17	0.12	0.15	0.35	0.38	0.22	0.24	0.25

The variation in R_F values of Fe^{3+}, Ag^+ and Pb^{2+} was found to be in the range of 0.05-0.11, 0.08-0.13 and 0.00-0.05 respectively depending upon the type of soil (S_1-S_8) used.
T refers to tailed spot (R_L - $R_T \geq 0.3$)

mobility ($R_F \cong 0.25$-0.38) was experienced when other fertilizers such as NH_4NO_3, NH_4Cl or urea were taken in the mobile phase (M_{24}, M_{23} or M_{15}). It shows that the mobility of Cu^{2+} is influenced by the nature of fertilizer.

(iv) In contrast to the behaviour of Cu^{2+}, Zn^{2+} which remained at or near the point of application in all fertilizers containing CTAB mobile phases showed an increased mobility ($R_F \cong 0.3$-0.57) when sodium phosphate was present in the mobile phase.

(v) Cd^{2+} showed peculiar behaviour with sodium phosphate as it gave double spots ($R_F \cong 0.14$-0.22 1st spot, $R_F \cong 0.69$-0.77 2nd spot) in all soil types used in the stationary phase with the exception of S_{11} and S_{16} layers where it gave elongated single ($R_F \cong 0.49$) and compact single ($R_F \cong 0.69$) spots respectively. The formation of double spots shows the presence of two species. It seems that the lower spot is due to Cd-phosphate complex whereas the upper spot is due to the free Cd^{2+} ions.

Separations achieved on different types of soil containing layers using M_{15} mobile phase are listed in Table 8. In most of the cases Cd^{2+} was easily separated from Zn^{2+} and Hg^{2+}. Quaternary separations were also achieved on almost all soil mixed with silica gel layers. Thus, it can be safely concluded that urea added aqueous cationic surfactant solutions are very useful mobile phases for separating heavy metal cations from their multicomponent mixtures. Most of the separations achieved were on soil containing silica mixed layers. The type of soil used in combination with silica influences the mobility as well as the detection clarity of metal cations. The soil mixed silica stationary phases can be used in the following order of preference to realize selected separation of metal cations:

Table 8—Experimentally achieved separations of metal ions on soil mixed with silica gel layers developed with mobile phase 0.5 M CTAB + 0.6 M urea (1:1).

Stationary phase	Separations (R_F)
S_9	Ni^{2+} (0.65) - Cu^{2+} (0.30) - Fe^{3+} (0.05), Ni^{2+} (0.62) - Cu^{2+} (0.30) - Pb^{2+} (0.00), Ni^{2+} (0.65) - Cu^{2+} (0.30) - Fe^{3+} (0.00) - Hg^{2+} (0.99), Zn^{2+} (0.12) - Cd^{2+} (0.60)-Hg^{2+} (0.95), Zn^{2+} (0.18)- Fe^{3+} (0.00) - Ni^{2+} (0.62) - Hg^{2+} (0.92).
S_{10}	Zn^{2+} (0.12) - Cd^{2+} (0.55) - Hg^{2+} (0.95), Ni^{2+} (0.7) - Fe^{3+} (0.03) - Hg^{2+} (0.94).
S_{11}	Ni^{2+} (0.72) - Cu^{2+} (0.21) - Fe^{3+} (0.05), Ni^{2+} (0.70) - Cu^{2+} (0.26) - Pb^{2+}(0.0), Fe^{3+} (0.02) - Cu^{2+} (0.24) - Ni^{2+} (0.68) - Hg^{2+} (0.97), Zn^{2+} (0.11) - Cd^{2+} (0.67) - Hg^{2+} (0.95), Zn^{2+} (0.17) - Fe^{3+} (0.0) - Ni^{2+} (0.63) - Hg^{2+} (0.95), Pb^{2+} (0.00)- Cd^{2+} (0.70) - Hg^{2+} (0.96).
S_{12}	Ni^{2+} (0.61) - Cu^{2+} (0.24) - Fe^{3+} (0.30), Fe^{3+} (0.02) - Cu^{2+} (0.31) - Hg^{2+} (0.99), Ni^{2+} (0.63) - Fe^{3+} (0.03) - Hg^{2+} (0.97), Fe^{3+} (0.02) - Cu^{2+} (0.24) - Ni^{2+} (0.63) - Hg^{2+} (0.97), Pb^{2+} (0.00) - Cu^{2+} (0.31) - Ni^{2+} (0.67) - Hg^{2+} (0.97).
S_{13}	Ni^{2+} (0.71) - Cu^{2+} (0.32) - Hg^{2+} (0.97), Fe^{3+} (0.03) - Cu^{2+} (0.33) - Hg^{2+} (0.97), Fe^{2+} (0.03) - Ni^{2+} (0.68) - Hg^{2+} (0.96), Fe^{2+} (0.02) - Cu^{2+} (0.32) - Ni^{2+} (0.72) - Hg^{2+} (0.97), Zn^{2+} (0.15) - Cd^{2+} (0.65) - Hg^{2+} (0.98), Pb^{2+} (0.00) - Cu^{2+} (0.31) - Ni^{2+} (0.71) - Hg^{2+} (0.97).
S_{14}	Fe^{3+}(0.05) - Cu^{2+} (0.25) - Ni^{2+} (0.65), Fe^{3+} (0.02) - Cu^{2+} (0.35) - Ni^{2+} (0.65) - Hg^{2+} (0.97), Zn^{2+} (0.14) - Cd^{2+} (0.67) - Hg^{2+} (0.96), Pb^{2+} (0.00) - Cu^{2+} (0.32) - Ni^{2+} (0.71) - Hg^{2+} (0.97).
S_{15}	Fe^{3+} (0.02) - Cu^{2+} (0.27) - Ni^{2+} (0.67), Fe^{3+} (0.02) - Cu^{2+} (0.25) - Hg^{2+} (0.95), Fe^{3+} (0.02) -Cu^{2+} (0.26) - Ni^{2+} (0.71) - Hg^{2+} (0.97), Zn^{2+} (0.12) - Cd^{2+} (0.60) - Hg^{2+} (0.91), Fe^{3+} (0.00) - Ni^{2+} (0.66)-Hg^{2+} (0.95), Pb^{2+} (0.00) - Cu^{2+} (0.27) - Ni^{2+} (0.61) - Hg^{2+} (0.95).
S_{16}	Zn^{2+} (0.11) - Cd^{2+} (0.58) - Hg^{2+} (0.98), Fe^{3+} (0.04) - Ni^{2+} (0.60) - Hg^{2+} (0.94), Pb^{2+} (0.00) - Ni^{2+} (0.65) - Hg^{2+} (0.97).

$S_{11}=S_{13}=S_{15}> S_9=S_{12}>S_{14}>S_{16}>S_{10}$

Since the mobility of metal cation was found to depend on the degree of silica gel present in the mixed bed containing soil, the results obtained have been summarized in Fig. 1. The following trends are noticeable:

(a) Increasing percentage of silica gel in the stationary phase has little effect on the mobility of Hg^{2+}.
(b) The mobility of Cd^{2+} and Ni^{2+} increases with the increase in silica gel content in the stationary phase.
(c) Fe^{3+} remains near the point of application, while Zn^{2+}, Pb^{2+} Ag^{+} and Bi^{3+} show very little mobility at all concentration levels of silica gel.
(d) Detection of Cu^{2+} and Co^{2+} becomes very difficult when the amount of soil is exceeded to 50% in the mixed layer i.e. soil plus silica gel mixture.

These results are important as the soil contaminated with silica gel allows the passage of Ni^{2+} and Cd^{2+} in addition to the transportation of Hg^{2+}, from the soil surface deep into the soil bed. The migration of these metal ions from soil surface may be harmful to vegetation because of their accumulation into plants through plant roots. Therefore, to avoid the toxic effect of Cd^{2+} and Ni^{2+}, the soil surface may be kept free from silica gel.

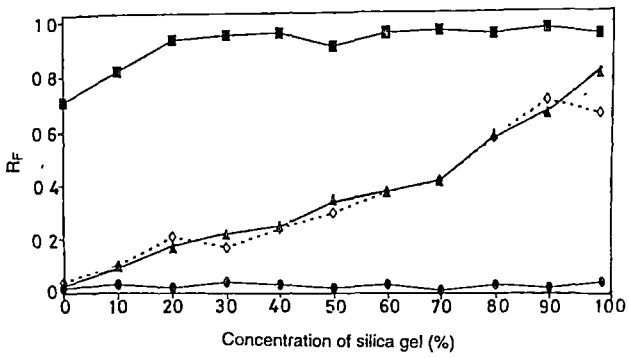

Fig. 1—Effect on the mobility of certain metal ions by the amount of silica gel present in the mixed stationary phase containing soil ◊---Cd, —■— Hg, —▲— Ni and —●— Fe

One of the interesting aspects of the present study is the salting – in effect shown by Cd^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Co^{2+}, Ag^{+} and Zn^{2+}. In such cases the R_M value ($R_M = 1-R_F/R_F$) decreases with the increase in ammonium sulphate concentration in distilled water.

To compare the chromatographic performance of mixed layer materials, the silica gel was replaced by alumina, cellulose, kieselguhr and flyash in the mixture of soil and silica gel (1:9) and the TLC plates coated with these layer materials were used to determine the mobility of metal ions using M_{15} mobile phase. None of the mixed adsorbents matches the performance of silica containing soil mixed layer. In case of flyash, the detection of metal ions was very difficult whereas in the cases of cellulose, alumina

and kieselguhr certain metal ions produced tailed spots.

Reproducibility defined as the precision under different conditions such as different analyses and different days is an important validation parameter. The variation in R_F values measured by three independent analyses and by the same analyst in different days did not differ by more than 0.15 (i.e. ±15%) from the average R_F value, indicating a good reproducibility.

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Internet address: http://www.us.edu.pl/uniwersytet/jednostki/wydzialy/chemia/acta/tekst/index.html

Katowice, February 19th, 2003

Prof. Dr. Ali Mohammad
Department of Applied Chemistry
Faculty of Engineering & Technology
Aligarh Muslim University
Aligarh-202 002, INDIA

Dear Professor Mohammad,

I am very pleased to be able to let you know that the submitted paper, its title:

'*Reversed-phase chromatography of amines, phenols and metal cations on silica layer impregnated with tributyl phosphate using surfactant-mediated mobile phase systems*'
(Authors: Ali Mohammad and N. Jabeen)

has been carefully scrutinized by our Reviewers and unanimously declared as valid for publication in *Acta Chromatographica*. Moreover, it was already scheduled for the No.13 / 2003 issue thereof, to appear from press some time in mid-2003.

Taking advantage of the opportunity, I would also like to thankfully address your choice of *Acta Chromatographica* as a suitable forum for publication of your most interesting and always perfectly presented scientific results.

With best regards,
yours sincerely,

Teresa Kowalska

Teresa Kowalska